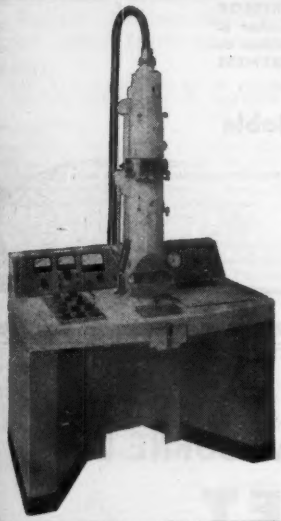
NATURE
A Weekly Journal of Science

UNIVERSITY OF MICHIGAN
FEB 21 1958
PERIODICAL READING ROOM

Vol. 181, No. 4604 SATURDAY, JANUARY 25, 1958 2s. 6d.



AT YOUR SERVICE —

— a fully equipped electron microscope laboratory staffed by experienced operators

Write for details and charges to:—

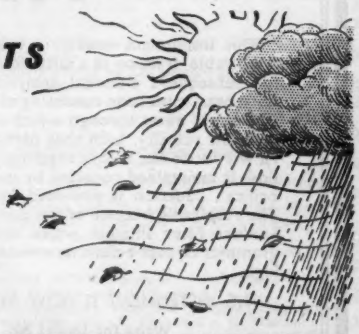
SCIENTIFIC APPARATUS DEPT.

METROPOLITAN-VICKERS
ELECTRICAL CO LTD · TRAFFORD PARK · MANCHESTER 17

An A.E.I. Company

N/R701

METEOROLOGICAL INSTRUMENTS



You will find the accuracy and robust construction you need in all meteorological instruments by Negretti & Zambra. Great sensitivity and long life are built-in qualities of every instrument we make.

Send for publication M/4, post free on request

NEGRETTI & ZAMBRA
122 REGENT STREET · LONDON W1
Telegrams: Negretti, Piccy, London. REGent 3406

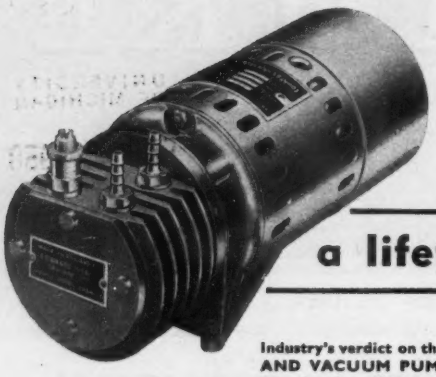
BRANCHES: BIRMINGHAM · CARDIFF · GLASGOW
LEEDS · MANCHESTER · NOTTINGHAM

TECHNICAL SERVICE AVAILABLE IN MOST COUNTRIES

ANEMOMETERS · BAROMETERS
HYGROMETERS · RAIN GAUGES
THERMOMETERS · SUNSHINE RECORDERS, etc.

Manufacturers of instruments for the indication, recording and controlling of temperature, pressure, liquid level, volume, specific gravity, humidity etc., aircraft instruments.

R.B Series . . .



"SPEEDIVAC"
COMBINED
VACUUM
and PRESSURE
UNITS

Vacuum
100 mms mercury (26" Hg)
125 mms mercury (25" Hg)
250 mms mercury (20" Hg)

Pressure
10 lb./sq. in. (0.7 kg./sq. cm.)
10 lb./sq. in. (0·7 kg./sq. cm.)
8 lb./sq. in. (0·56 kg./sq. cm.)

a lifetime of DOUBLE service...

Industry's verdict on the "SPEEDIVAC" ROTARY COMPRESSOR AND VACUUM PUMP is clearly expressed in the number of *repeat* orders placed and wholeheartedly endorses our claims to QUIET EFFICIENCY - . . COMPACTNESS . . . ROBUSTNESS . . UNFAILING SERVICE.

Several alternative models available

For better vacμμm service . . .

EDWARDS HIGH VACUUM LTD

MANOR ROYAL · CRAWLEY · SUSSEX CRAWLEY 1500 (10 LINES) EDCOHIVAC CRAWLEY

CASELLA LONDON

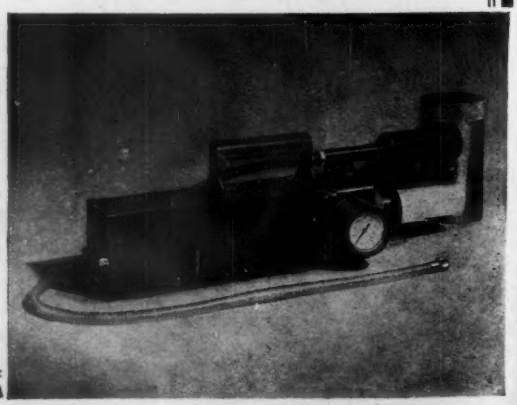
A NEW INSTRUMENT FOR SAMPLING AIRBORNE DUST—

THE HEXHLET

This instrument enables a bulk sample of the 'respirable' fraction of a dust cloud to be collected for subsequent chemical analysis. It employs a horizontal elutriator consisting of a number of flat horizontal ducts through which air is drawn at a constant velocity, such that particles above about 7μ will settle out before reaching the end. The air flow is maintained constant by means of a 'critical orifice'. Suction is provided by an air ejector. The respirable fraction of the dust is collected in a Soxhlet filter thimble which will hold up to 2 grammes of dust before its resistance becomes too high.

THE INSTRUMENT IS NOW IN PRODUCTION

Write for Leaflet No. 865



C. F. CASELLA & Co. Ltd
REGENT HOUSE, FITZROY SQUARE
LONDON, W.1
Telephone : ★EUSton 3944
Member Scientific Instrument Manufacturers' Association of Great Britain

NATURE

No. 4604 SATURDAY, JANUARY 25, 1958 Vol. 181

CONTENTS

Page

Harnessing Nuclear Fusion . . . 213

Enigmatic Eddington. By Prof. W. H. McCrea, F.R.S. . . . 214
Hydrography. By Dr. G. E. R. Deacon, C.B.E., F.R.S. . . . 214
Prehistoric Denmark. By M. C. Burkitt . . . 215
The Biochemical Lesion. By Dr. C. P. Stewart . . . 215
Mechanism in Behaviour. By Dr. J. T. Eayrs . . . 216

CONTROLLED RELEASE OF THERMONUCLEAR ENERGY . . . 217

Contributions from :
The Atomic Energy Research Establishment, Harwell
Princeton University
Research Laboratory, Associated Electrical Industries, Ltd., Aldermaston
Los Alamos Scientific Laboratory, University of California

Obituaries :
Prof. M. A. Catalán. By Dr. R. Velasco . . . 234
Prof. Louis Pillemer. By Dr. Bernhard Cinader . . . 234

News and Views . . . 235

Newer Preparative Methods in Organic Chemistry . . . 239
Interaction in Ionic Solutions. By Dr. J. E. Prue . . . 240
Reorganization of the Meteorological Office . . . 241
Rothamsted Experimental Station. By R. M. S. Perrin . . . 242
Astronomy at the Cape of Good Hope . . . 243
Linnæus. By Dr. J. Ramsbottom, O.B.E. . . . 244
Applied Probability. By D. G. Kendall . . . 245
Use of Faradaic Rectification for the Study of Rapid Electrode Processes. By Dr. G. C. Barker, R. L. Faircloth and A. W. Gardner . . . 247
Constitution of the Fibrillar and Non-fibrillar Components of the Walls of *Valonia ventricosa*. By Prof. R. D. Preston, F.R.S., and J. Cronshaw 248
Inhibition of Carotenoid Synthesis in Photosynthetic Bacteria. By Germaine Cohen-Bazire and Prof. R. Y. Stanier ; Dr. R. C. Fuller and I. C. Anderson . . . 250
Natural Occurrence of Thymine and Three Methylated Adenine Bases in Several Ribonucleic Acids. By Dr. J. W. Littlefield and Dr. D. B. Dunn 254

Letters to the Editors :

Iodine-131 in Sheep before and after a Nuclear Reactor Accident.—Prof. L. Van Middlesworth . . . 256
Symmetry of Snow Crystals.—Prof. S. Tolansky, F.R.S. . . . 256
A Photographic Observation of the Satellite 1957 Beta leaving the Earth's Shadow.—Dr. Heinz Neckel . . . 257
Precision Determination of the Velocity of Electromagnetic Waves.—K. D. Froome . . . 258
Metallographic Studies of Iron Carbide and Tungsten Carbide.—F. J. Radd and L. H. Crowder . . . 258
Two Borides with the Cementite Structure.—Stig Rundqvist . . . 259
Amplitude of a Quartz Plate vibrating in Liquids.—Dr. S. Parthasarathy and Harkrishan Singh . . . 260
Surface Hardness of Explosive Materials by Micro-indentation.—Dr. G. Todd and E. Parry . . . 260
Vapour Pressure of Tritiated Water.—A. H. Price . . . 262
Use of Gaussian Functions for Intermolecular Potentials.—Dr. K. Singer . . . 262
Improved Resolution of X-Ray Diffraction Patterns of Protein Crystals at Low Temperature.—Dr. Murray Vernon King . . . 263
Separation of the Lower Fatty Acids (C_1 to C_{10}) by High-Voltage Paper Electrophoresis.—Dr. D. Gross . . . 264
The Basicities of N-Alkyl Anilines.—H. T. Taylor . . . 265
Infra-red Absorption Spectra of Casein and Lactose.—K. P. Norris and J. E. S. Greenstreet . . . 265
Effects of Crystallinity on the Infra-red Absorption Spectra of Lactose and Dried Milk.—J. D. S. Goulden and Jonathan W. White, jun. . . . 266
'Multiple' Hæmoglobins in the Horse.—Dr. A. D. Bangham and Dr. H. Lehmann . . . 267
The Diego Blood Group System.—Dr. Bruce Chown, Marion Lewis and Hiroko Kaita. . . . 268
Separation of Carotenoid Synthesis from Fat Synthesis in *Rhodotorula gracilis* by Diphenylamine.—L. Slechta, O. Gabriel and Dr. O. Hoffmann-Ostenhof . . . 268
Preparation of Transparent Starch Gels after Electrophoresis.—Dr. J. M. Fine and E. Waszczenko-Z . . . 269
Reversal of Thymine Xyloside Inhibition in *Escherichia coli* by Glutathione.—Prof. Thomas D. Brock . . . 269
Aerobic Inhibition of Glycolysis.—J. F. Turner and Dr. L. W. Mapson . . . 270
Formation of Histamine-containing Dinucleotides by Enzymatic Interaction of Histamine and Coenzyme I—Dr. Spyridon G. A. Alivisatos . . . 271
Accumulation of α-Alanine and of Pyruvate in Diphtherial Culture Filtrate and its Relation to Iron Deficiency of Medium.—Prof. Masahiko Yoneda and Hiroshi Ishihara . . . 272
Thyroxine Metabolism by Extracts of Rat Liver.—Lawrence G. Piaskett . . . 273
The Biuret Complex of Gelatin and the Mechanism of Gelation.—J. Bello and J. R. Vinograd . . . 273
Autoradiographic Visualization of Synthesis of Deoxyribonucleic Acid in Tissue Culture with Tritium-labelled Thymidine.—H. Firket and W. G. Verly . . . 274
Reduction of Low-Temperature Injury to Stored Apples by Modulation of Environmental Conditions.—W. Hugh Smith . . . 275
A Staining Technique for detecting Ratoon Stunting Disease in Sugar Cane.—Robert Antoine . . . 276
Occurrence of a Blue-Green Alga in the Nodules of *Trifolium alexandrinum*.—S. Bhaskaran and G. S. Venkataraman . . . 277
Agar Double Diffusion Precipitation Reaction applied to the Study of *Asterococcus mycoides*.—G. White . . . 278
Incompatibility in *Theobroma cacao*.—F. W. Cope . . . 279
A Chromatographic Method for studying Simple Compounds in Plants.—R. N. Greenshields . . . 280
Fluorescent Substances produced by Dermatophytes.—Dr. F. W. Chattaway and A. J. E. Barlow . . . 281
Streptomycin and Bacterial Respiration.—F. L. Jackson . . . 281
Inhibition of Blood Digestion in Mosquitoes by Cations, and Cation-Antibiotic Mixtures.—Dr. Levon A. Terzian . . . 282
Response of the Adrenal Medulla of the Sheep Fœtus to Asphyxia.—Dr. R. S. Comline and Marian Silver . . . 283
Effect of Two Extreme Temperatures on Growth and Tail-length of Mice.—Dr. M. A. El-R. Ashoub . . . 284
An Acid-fast Bacillus Isolated from the Lungs of the Cape Hyrax, *Procavia capensis* (Pallas).—J. C. Wagner, Dr. G. Buchanan, V. Bokkenheuser and Shirley Leviseur . . . 284
Microbial Growth Stimulants in Spleen.—Dr. M. L. Littman and Dr. Michael A. Pisano . . . 285
Amino-acid Composition of Sclero-protein of the Sponge *Hippospongia equina*.—Dr. J. Saper and W. E. White . . . 285
Fluorescence of the Crystalline Lens and Electroretinographic Sensitivity Determinations.—Dr. E. Dodt and J. B. Walther . . . 286
Effect of a Raised Peripheral Temperature upon the Rate of Regeneration in a Mammalian Peripheral Nerve.—H. J. Gamble . . . 287
A Method for studying separately the Properties of the Innervated and Non-innervated Membrane of an isolated Single Electroplax of the Skate.—Dr. Ernest Schoffeniels . . . 287
Effect of Periodic Acid on Histochemical Aldehyde Blockades.—Dr. P. H. Staple . . . 288
A Quantitative Study of Stasigenesis in Fissipede Carnivores.—Dr. M. Crusafont-Pairó and Dr. J. Truyols-Santonja . . . 289
Induction of Triploidy in the South African Clawed Frog, *Xenopus laevis* (Daudin).—Sheila Smith . . . 290
Epimorphic Regeneration during Hibernation.—Timothy J. Hunt 290

Recent Scientific and Technical Books . . . Supp. viii

CHAPMAN & HALL

★ *Just Published* ★

ORGANIC ELECTRODE PROCESSES

by

Milton J. Allen

Director of the Physical Research Laboratories, CIBA Pharmaceutical Products Inc., Summit, N.J.

The author has devoted his whole career to organic electro-chemical investigations involving methods of preparation of organic compounds utilising oxidation or reduction techniques. His studies have also encompassed the mechanisms and kinetics of organic electrode processes. Mr. Allen's purpose in writing his book is to give organic chemists the fundamental information to start investigatory work.

Size: 9½ in. × 6 in. 216 *pages* *Illustrated* 52*s. net*

CHEMISTRY OF NATURAL AND SYNTHETIC RUBBERS

by

Harry L. Fisher

Professor of Chemical Engineering, University of S. California

A REINHOLD BOOK

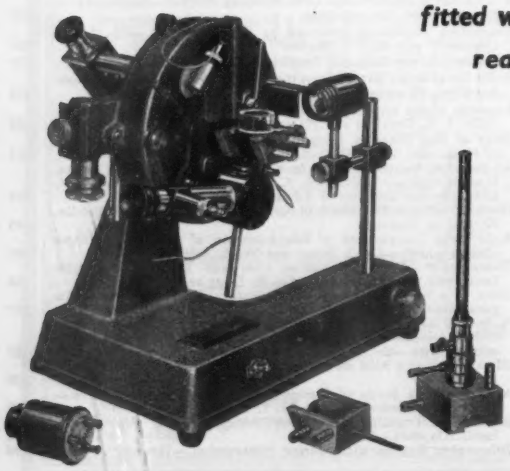
A comprehensive survey of the subject. It covers: Elastomers: History & Chemistry—Vulcanization—Acceleration—Antioxidation and Antiiozonation—Natural Rubber—Latex—Properties of Natural and Synthetic Rubbers—Some Raw Materials for Synthetic Rubbers—Synthetic Rubbers—Hard Rubbers—Bonding Rubber to Metal—Reclaimed Rubber—Chemical Derivatives.

Size: 9 in. × 6 in. 188 *pages* 32*s. net*

37 ESSEX STREET, LONDON, W.C.2

PRECISION BS INSTRUMENTS

Pulfrich Refractometer



fitted with circle divided on glass and giving readings on opposite sides of the circle to 5 seconds, by double microscope

This instrument possesses many advantages over the older type of Pulfrich refractometer and is used in all parts of the world for testing oils, optical glass, chemicals, drugs, etc.

We shall be pleased to demonstrate this instrument and give any advice as to its use, by appointment at our Works.

Bellingham & Stanley Ltd.

DEPT. N, 71 HORNSEY RISE, LONDON, N.19

PHONE: ARCHWAY 2270

HARNESSING NUCLEAR FUSION

IN replying on November 11 to a question in the House of Commons, the Paymaster-General, speaking for the Government, said that the experimental apparatus known as ZETA at the Atomic Energy Research Establishment, Harwell, was operating successfully, and discharges through heavy hydrogen were giving very high temperatures, accompanied by discharges of neutrons, believed to be due to thermonuclear reactions. It is appropriate that the first detailed account of this work should appear in *Nature*, where it will be read by scientists all over the world (see p. 217 of this issue).

Ever since the possibility of nuclear fission was realized, it was understood that a converse process of fusion of atomic nuclei was possible and would yield a vast amount of energy. This possibility became a fact with the invention of the hydrogen bomb. All the time, however, scientists in many countries were endeavouring to learn more about the mechanism of the reaction with the view of controlling this immense explosive force. A carefully considered account, by one of the Harwell scientists, of the theory involved was published in *Nature* of May 4, 1957, p. 886; this demonstrated the very stringent physical conditions which control of such processes must entail. Although it is generally recognized that we are continually witnessing the results of thermonuclear reactions—for they are believed to be the source of the heat and light given out by the Sun—their production and control in the laboratory present problems hitherto unparalleled.

The communications from British and American scientists appearing in this issue of *Nature* demonstrate that the first steps in this great task have been successfully taken. Teams of scientists at Harwell and Aldermaston have obtained results which led to the brief announcement made in the House of Commons on November 11. American scientists, working on slightly different lines, have also contributed their results, and it is heartening to read the tribute they pay to the Harwell scientists.

Bearing in mind the scientific importance and wide interest of the work already done, the lag between the Government's brief statement and the present publication of British and American results suggests that difficulties other than those of the laboratory have been met. The work was being done, perhaps inevitably in view of its complexity, under government auspices, in the two countries, and was subject to security restrictions. There seems to have been considerable exchange of information between the scientists directly concerned; but it is regrettable that security measures should have been invoked in such a quest, the significance of which for military purposes would not appear to be greater than that of innumerable scientific investigations in progress in various laboratories in both countries. Only those with full knowledge of the situation can give a final answer to this question.

However, the announcement of the successful control of the thermonuclear reaction has now been made, and all the scientists and others concerned are to be congratulated on their magnificent achievement.

Under the stress of military needs during the Second World War, great efforts were concentrated on the production of the atom bomb, leading to the preparation of fissile material of various kinds; the problem of converting this weapon to the production of energy for peaceful purposes was, for the time being, in the background. But with the end of open warfare, its utilization for the production of useful power became an urgent problem. The culmination of this effort was seen in the opening by H.M. The Queen in 1956 of Calder Hall power station, the first in the world to be solely dependent on atomic energy as a source of power. But the use of fissile material for this purpose, though a triumph of scientific and engineering skill, brought its own difficulties in the economic and other fields, for the initial cost of atomic fuel is high and it is a scarce commodity. Atomic energy power stations and the conventional power stations were seen to be complementary rather than competitive. Now we have the possibility of utilizing another 'atomic' fuel, of wonderful potentiality and world-wide occurrence, though whether it becomes the basis of the generation of electrical power, or means are found of making direct use of it as a source of energy, only the future will show. Meanwhile the control of nuclear fusion will be welcomed both as a great advance in science and as a factor of tremendous importance for the future, bringing vast stores of energy into the service of man for his use in the amelioration of his conditions.

In 1831, Michael Faraday discovered electromagnetic induction. His apparatus was of the simplest kind, consisting of a ring of iron on which two coils of wire insulated with "twine and calico" were wound, and a compass needle as detector. The experiment was not a chance affair, for his thoughts had turned repeatedly to the subject, and he had tried some experiments, but without success. Then he hit upon the correct sequence of events, and the experiment succeeded. Electricity had been generated in one simple circuit by making and breaking another simple circuit. From that experiment the electrical industry was born—the electrical age was initiated; and although its growth was at first slow, it now dominates the industrial world.

The new work on thermonuclear reactions is at present a laboratory experiment, albeit with most complex and costly equipment and even the risk of dangerous irradiation; it is clearly a first step, even as Faraday's experiment was. Life on the Earth has always depended on nuclear reactions in the Sun, and now it seems that man is learning to reproduce those reactions in the laboratory. Are we witnessing the birth of a new age—that of the utilization of man-made thermonuclear reactions?

ENIGMATIC EDDINGTON

The Development and Meaning of Eddington's 'Fundamental Theory'

Including a Compilation from Eddington's Unpublished Manuscripts. By Dr. Noel B. Slater. Pp. xii+300. (Cambridge: At the University Press, 1957.) 40*s*. net.

EDDINGTON regarded physics as the structural knowledge of measures of an external world of objects that can be indicated but the intrinsic nature of which is not discoverable. Fundamental physics was for him the part concerned with properties of measures simply as operations, independently of what they operate on. He claimed to have discovered that fundamental physics in this sense comprises all the general laws of Nature, including the evaluation of all the constants involved. His book, "Fundamental Theory" (Cambridge 1946), was the final account of this work. The manuscript was not quite complete when he died in 1944, but was printed as he had left it, under the editorship of the late Sir Edmund Whittaker (who included one of Eddington's published papers on the intended subject of the missing last chapter).

Large parts of five or six earlier drafts had been preserved by Eddington, and Dr. N. B. Slater has collated these in the present book. After summaries of the main sections of "Fundamental Theory", Dr. Slater gives notes on the various drafts and extensive quotations wherever he considers that they contain a significant variant of an argument or material omitted from the final version. He provides a bibliography of writings on the subject that have been of assistance in preparing the book, and a useful list of the symbols employed by Eddington. He has also noted an error in a statistical argument of Eddington's that calls for one or two amendments in the evaluation of certain constants. His task has been extremely onerous, all the more so because the material reached him piecemeal in the course of the work. Clearly, he has exercised the utmost thoroughness, and the plan he has followed is almost certainly the one making for greatest conciseness. Dr. Slater is not to be blamed for the one regrettable feature of the presentation, the smallness of the type used for all quotations from Eddington.

Most physicists would go some way with Eddington in recognizing that their results are not so much properties of an external world as properties of their own operations upon such a world. A number would be glad to be able to go further. Each major conclusion as Eddington describes it in general terms appeals to them as possessing a high degree of plausibility and, if valid, as being profoundly illuminating. Yet they find insuperable difficulty in seeing very much connexion between any such conclusion and the detailed arguments and calculations that purport to establish it. A few physicists are, indeed, prepared to follow Eddington through separate sections of the work but without being satisfied as to the connexions between the sections. So far as I am aware, however, no physicist professes to understand the arguments of "Fundamental Theory" sufficiently thoroughly to be able to assert or deny that it provides a continuous chain of reasoning.

Were Eddington's work acceptable, it would of course be one of the very great advances in scientific thought. The new book might have led to a better understanding of the work had the available material shown how Eddington proceeded from an early stage to build up his final intricate structure. As Dr. Slater remarks, however, almost all the material at his disposal had been intended as a final version when it was written. One cannot, therefore, see much hope that it will help substantially to resolve the enigma of "Fundamental Theory".

The new material, together with Dr. Slater's commentaries, does make clearer than before what Eddington was trying to achieve. Also it shows that Eddington was wonderfully confident that he had achieved it. One cannot help thinking that he has at least indicated the general character of probable developments in physical theory. Such developments may ultimately come from some completely independent approach, or they may come from work like that of E. W. Bastin and C. W. Kilmister that is probably to some extent inspired by Eddington. But one would hazard the view that they are now scarcely likely to flow directly from Eddington's own treatment. For until acceptable conclusions have been reached by other means, it will probably be impossible to know how far Eddington relied upon his astonishing power of intuition. Nevertheless, Eddington's thought never lost its quality of greatness, even when it was most enigmatic, and we must be grateful to Dr. Slater and the publishers for this additional record of it.

W. H. McCrea

HYDROGRAPHY

Hydrography of the Faroe–Shetland Channel, 1927–1952

By Dr. John B. Tait. (Scottish Home Department. Marine Research, 1957, No. 2.) Pp. ix+309. (Edinburgh and London: H.M. Stationery Office, 1957.) 105*s*. net.

FISHERY scientists have good reason to believe that changes in ocean currents and water circulation have an important bearing on variations in local stocks of fish and migration of fish shoals. For the whole of north-west Europe it is important to have a good knowledge of the varying balance between Arctic and Atlantic influences, and for the North Sea itself we must understand the varying influence of oceanic water which mixes with coastal water and flows from north to south across the herring fishing grounds. The first essential is to have measurements of what has taken place, and the most promising method known so far is to make regular surveys of the changing temperature and salinity of the water. The Faroe–Shetland Channel is a particularly informative area because a large proportion of the Atlantic water that warms the northern North Sea and the Arctic Ocean passes through it.

Dr. Tait's large monograph gives a detailed presentation of the vertical distribution of temperature and salinity along two roughly parallel cross-sections from the Faroe Islands to the Shetland Islands and from the Faroe Bank to the Butt of Lewis. On twenty-two occasions between 1927 and 1952 observations were made along both lines, and on a further twenty-five they were made along one or the other. Every now and then other lines were worked. The monograph gives diagrams showing the distribution of temperature and salinity in the vertical plane for every line of observations, and the observations themselves are listed in Appendix 2. It also

gives the computed transport of water across each line assuming geostrophic flow and the existence of little or no current at the depth of the 35 parts per 1,000 isohaline. These assumptions are the best that can be made until it is feasible to carry out a very large programme of deep-current measurements, and they are supported by good general agreement between conclusions based on the two lines 120 miles apart, and by a few current measurements described in Appendix 1.

The work reveals large seasonal and long-term variations in the north-eastward transport of water, and opens up large and fascinating fields of research into the energy exchanges and hydrodynamical processes that cause them and into their effect on the complex hydrographic conditions and biology of the area. Linked with the main problem there is much information about the different types of water that are encountered there and practical conclusions as to their origin and formation. Enormous numbers of observations have been made with great care under difficult conditions, and this thorough and detailed study is a work of great value. It is prefaced by a good historical introduction, and it will itself be the foundation of much future work as well as serving present needs. G. E. R. DEACON

PREHISTORIC DENMARK

Denmark Before the Vikings

By Dr. Ole Klindt-Jensen. (Ancient Peoples and Places.) Pp. 212 (73 plates). (London: Thames and Hudson, Ltd., 1957.) 21*s.* net.

DENMARK is a small country, but it has bred noted archæologists. As long ago as 1836 Christian Thomsen of the National Museum suggested the well-known triple subdivision of human history into the ages of Stone, Bronze and Iron. Later the names of Worsaae and Sophus Müller became world renowned. Denmark is rich in prehistoric remains and much exploration work has been done there, but no short, satisfactory account of the prehistory of the country has been published for very many years.

The new volume on Denmark by Dr. O. Klindt-Jensen, who is the curator at the National Museum in Copenhagen, is therefore very welcome. After a brief introduction there is a chapter headed "The Palæolithic". But while the term Palæolithic is not entirely inaccurate since some of the earliest cultures found in the country do, like that of Hamburg, overlap with the end of Magdalenian times, there is nothing in these northern regions to compare with the rich upper Palæolithic cultures of France and northern Spain with their wealth of industries in stone and bone and their superb art, both in the prehistoric homes and on the walls of caves. The industries of these northern cultures are at first simple, but there soon appear others belonging to the so-called point cultures which stretched westwards from as far away as Poland, where they occur below some ancient dunes. Later the classic Mesolithic cultures are represented at Maglemosean sites and in the Kitchen Middens. Actually the earliest manifestation of the former (Maglemose) culture is perhaps best seen in Britain at Star Carr, but in Denmark there are many sites and much exploration has taken place. It may be added that digging a late Mesolithic (Kitchen Midden) site is no fun—the fine white dust penetrates everywhere and everything. Denmark is particularly rich in Neolithic remains of all kinds; not least there is a magnificent series of Megalithic monuments. The author's account of these is necessarily brief, but gives a good picture of the situation. Chapters on the Bronze Age up to the Dark Ages follow, and there is a concluding chapter on the prehistoric landscape. Following some notes and a short bibliography there come the plates—no less than 73, occupying 48 full pages. These are well chosen and well illustrate the text.

Doubtless the specialist in one or more of the periods covered will complain that too much has had to be left out. But the ordinary reader will congratulate the general editor of the series, Dr. Glyn Daniel, on having persuaded Dr. Klindt-Jensen to undertake the work, and the author himself for having produced such an excellent little volume. M. C. BURKITT

THE BIOCHEMICAL LESION

Biochemical Disorders in Human Disease

Edited by Prof. R. H. S. Thompson and Prof. E. J. King. Pp. xiv+843. (London: J. and A. Churchill, Ltd.; New York: Academic Press, Inc., 1957.) 90*s.*

THE enormous strides made by biochemistry during the past half-century and the ever-increasing application of biochemical concepts, as well as biochemical techniques, to the study of disease, have made important additions to the points of view from which disease can be surveyed, described and examined. From the cellular, we have advanced to the molecular level, and we can now think both of the anatomical or histological lesion and the underlying, more fundamental, 'biochemical lesion'. This convenient term, which we owe to Sir Rudolph Peters, is not actually used by the editors in the preface of this book, but it is implicit in what they say of their aims and lines of approach, and is indeed employed by contributors.

The object of the editors in planning the book was, for the benefit of both physicians and biochemists, to "assemble, relate, and interpret the known facts concerning the biochemical disorders that underlie or are associated with human disease"; the method was to ask each of a number of acknowledged experts to "describe . . . how the various diseases of any given organic system are dependent on biochemical disorders and to indicate the trends of biochemical work now in progress . . .". It can be said at once that the method has proved admirably adapted to a laudable aim. The editors and their fellow-contributors have succeeded in producing an exceedingly valuable addition to medico-biochemical literature and one which, I suspect, is likely to prove the prototype of the 'text-book of medicine' for the next generation.

Each author has been left free to interpret the editors' general directive in his own way, and as a result there is a considerable variety of treatment. This does not necessarily reflect differences in personal outlook, for the subjects are not equally 'advanced' from the biochemical point of view. In every case, however, the interpretation has clearly been that involved in the title of the book and in that of this review. In the past there has been too much emphasis on the function of biochemistry applied to medicine as a provider of diagnostic and prognostic tools, too little emphasis on its use in

describing and explaining the nature and development of the disease processes; yet the latter, which indeed includes, and is a logical extension of, the former, is by far the more important function of 'clinical chemistry' or 'chemical pathology'. In these chapters the diagnostic aids are discussed fully and extremely clearly, not as ends in themselves, nor as helping the physician to tie an appropriate label on his individual patients, but as the means of investigating the fundamental abnormalities whether considered as a general state covered by the label or as they afflict the individual. This is an essentially modern approach, well calculated to lead to a real understanding of disease and, through that, to therapy which can be adjusted rationally to the changing needs of a particular case.

The arrangement of the book is logical in relation to its general theme and it is a remarkable tribute to contributors and editors alike that the various chapters, written by different authors and each a review capable of standing alone, cover the ground fully, connectedly, yet without unnecessary overlapping. Twenty chapters deal with the diseases of the major organs (gastrointestinal tract, liver, kidney, pancreas, major endocrine glands, muscle), with disease groups not referable to particular organs (anæmias, hypertension, etc.) and with suitably grouped metabolic disorders (diseases of nutrition, porphyrias, lipoidosis and the like). Such an arrangement, probably the best for the clinician, means that a single biochemical topic such as acid-base equilibrium may be treated in several chapters by several authors and from various points of view. This is no disadvantage but is, indeed, one of the stimulating features of the book.

One ventures to predict that "Thompson and King" will become a classic, read and studied by senior medical undergraduates, by clinicians who wish to acquire understanding of their work, and by biochemists who, professionally or through interest, are concerned in the application of their science to the study of disease.

C. P. Stewart

MECHANISM IN BEHAVIOUR

Behavior Mechanisms in Monkeys

By Heinrich Klüver. Second impression. Pp. xvii+387. (Chicago, Ill.: University of Chicago Press; London: Cambridge University Press, 1957.) 49*s*. net.

THE study of the mechanisms underlying the activity of the central nervous system has claimed the attention of workers engaged in disciplines as far removed as, for example, psychology and electronic engineering, and it is therefore not surprising that there should be marked differences of emphasis and approach. Thus, of recent years, attention has been focused on mechanisms as they exist at the level of the individual cell, on the way in which neurones influence each other's activity, and on the part played by chemical processes in the specification and patterning of cerebral structure. Concurrently, and from quite a different point of view, mathematicians and communication engineers have been prompted to suggest that many problems of cerebral organization and function can best be treated on a statistical basis and to formulate hypotheses incorporating the established concepts of information theory and conditional probability to replace older views concerning the relationship between stimulus and response. To be fruitful, such advances in knowledge and thinking must be constantly applied to what is known about the reactions of the organism as a whole, and it is therefore particularly timely that Prof. Klüver's classic monograph "Behavior Mechanisms in Monkeys", which has been out of print for many years, has now been re-issued.

Prof. Klüver interprets the mechanism of behaviour in phenomenological terms, and his analysis is based on a study of those "general modes of reaction" from which it is possible "to predict the way an animal is going to react to a specific stimulus". He does not concern himself with the neural processes which underlie behaviour, but stresses, in his opening chapters, that little progress can be made in the investigation of cerebral function at the physiological level in the absence of firmly established experimental data concerning the overt factors governing the reactions of the individual to its environment. It is to this end that his monograph is directed.

The major portion of the book is devoted to a description and analysis of problems "selected chiefly because of their possible significance for neurophysiology". Experimental results are reported in considerable detail and fall into two groups. The first of these deals with investigations into the features of the stimulus situation to which the monkey responds. Variations of the 'pulling-in technique', which has since been extensively applied in experimental psychology, are used for determining the equivalence or non-equivalence of stimuli in the performance of discriminations involving weight, visual appearance and sound. The findings show an incidence of response to the relational aspects of the experimental situation which is consistently high over a wide range of cues; but there are occasions, particularly in the case of some visual discriminations, in which the animal responds to absolute sensory criteria. The second group of experiments deals in a similar way with such behavioural capacities as visual and tactual acuity, handedness and the ability to use common objects as tools.

Prof. Klüver ends with a thorough and critical analysis of his findings. He concludes that to account for the persistence of certain forms of discriminative behaviour in the face of marked changes in the environment, it is necessary to postulate the presence of some component which is identifiable throughout the range of experimental situations; but any definition of the property so abstracted as "an element which the stimuli have objectively in common" is unacceptable. Similarly, since the "response to relations" is not invariant, the unmodified concept of 'generalization' as a basic mechanism must be regarded as an over-simplification unless the individual characteristics of each stimulus situation are taken into account.

This book, which first appeared in 1933, is aimed at the serious student of behaviour rather than at the casual reader, and those unfamiliar with the extensive literature cited may have difficulty in following some of the arguments. Later developments are referred to in a new preface, but many of the original issues raised by Prof. Klüver still remain unresolved and it is gratifying that his monograph is once again readily available to a new generation of workers in the field of behavioural research.

J. T. Eayrs

CONTROLLED RELEASE OF THERMONUCLEAR ENERGY

PRODUCTION OF HIGH TEMPERATURES AND NUCLEAR REACTIONS IN A GAS DISCHARGE

By Dr. P. C. THONEMANN, E. P. BUTT, R. CARRUTHERS, Dr. A. N. DELLIS, D. W. FRY, Dr. A. GIBSON, G. N. HARDING, D. J. LEES, R. W. P. McWHIRTER, R. S. PEASE, Dr. S. A. RAMSDEN and S. WARD

Atomic Energy Research Establishment, Harwell

Introduction

THE basic conditions which must be established before a thermonuclear reactor is possible are, first, the containment of a high-temperature gas so that it is isolated from the walls of the surrounding vessel, and second, the attainment of temperatures sufficiently high for nuclear reactions to take place between the light elements. These two conditions are interdependent. Poor containment results in energy losses so large that gas temperatures much exceeding 10^6 °K. are unattainable.

The experimental apparatus described was designed to study the containment of ionized hydrogen (or deuterium) by the magnetic field associated with the current flowing in the gas and to reach temperatures sufficiently high for nuclear reactions to be detectable using deuterium. This apparatus, known as ZETA, was built at the Atomic Energy Research Establishment, Harwell.

The principles underlying the containment of a high-temperature gas by means of the 'pinch effect' have been discussed by a number of authors[1–3].

The constricted gas discharge formed by passing a high current through a low-pressure gas, while isolating the gas from the tube walls for short periods of time, rapidly develops instabilities and distortions which lead to bombardment of the walls by electrons and positive ions. The consequent cooling and recombination make it impossible to maintain high temperatures except for a transient period[4–8]. These instabilities can be suppressed by the combination of an axial magnetic field parallel to the direction of the discharge current and by the fields produced by eddy currents induced in the surrounding metal walls when the current channel changes its position.

Theoretical studies of discharge stability in the presence of an axial magnetic field have been published[9–12], together with experimental evidence for stability in a straight discharge tube[13].

The preliminary results reported in this article show that relatively long-time stability can be achieved in a toroidal metal-walled tube. The neutron yield and the kinetic ion temperatures have been measured over a limited range of conditions. The nuclear reaction-rates observed are not inconsistent with those expected from a thermonuclear process.

Apparatus

ZETA is a ring-shaped discharge tube of aluminium, 1-m. bore and 3-m. mean diameter, containing gas at low pressure. The gas, usually at a pressure of about 10^{-4} mm. of mercury, is made weakly conducting by a radio-frequency discharge. The toroidal ionized gas plasma forms the secondary of a large iron-cored pulse transformer. A condenser bank, storing up to a maximum of 5×10^5 joules, is discharged into the primary of the transformer, and produces a unidirectional current pulse in the gas up to a maximum of 200,000 amp. The current pulse in the gas lasts for about 4 msec. and is repeated every 10 sec. A steady axial magnetic field is generated by current-carrying coils wound on the torus. This field can be varied from zero to 400 gauss.

Fig. 1 shows the discharge tube assembled with the transformer. A vacuum spectrograph can be seen connected to the torus body.

Electrical Characteristics

Typical current and voltage oscillograms are shown in Fig. 2. The top trace is the 'voltage per turn' measured by a loop around the iron core enclosed by the discharge tube. The length of the discharge path is approximately 1,000 cm. so that the initial electric field at the boundary of the ionized gas is about 2 V. per cm. The primary winding is short-circuited when the voltage per turn is zero, thus preventing the charge on the condensers reversing. The second trace shows the current flowing in the gas, which persists for about 2 msec. after the transformer primary is short-circuited.

As the current decreases the plasma expands until it reaches the walls. It is then cooled. A sudden increase in resistance accompanies this process and the consequent increase in $|dI/dt|$ produces a severe voltage transient which may rise to tens of kilovolts. Destructive voltage transients of this type are

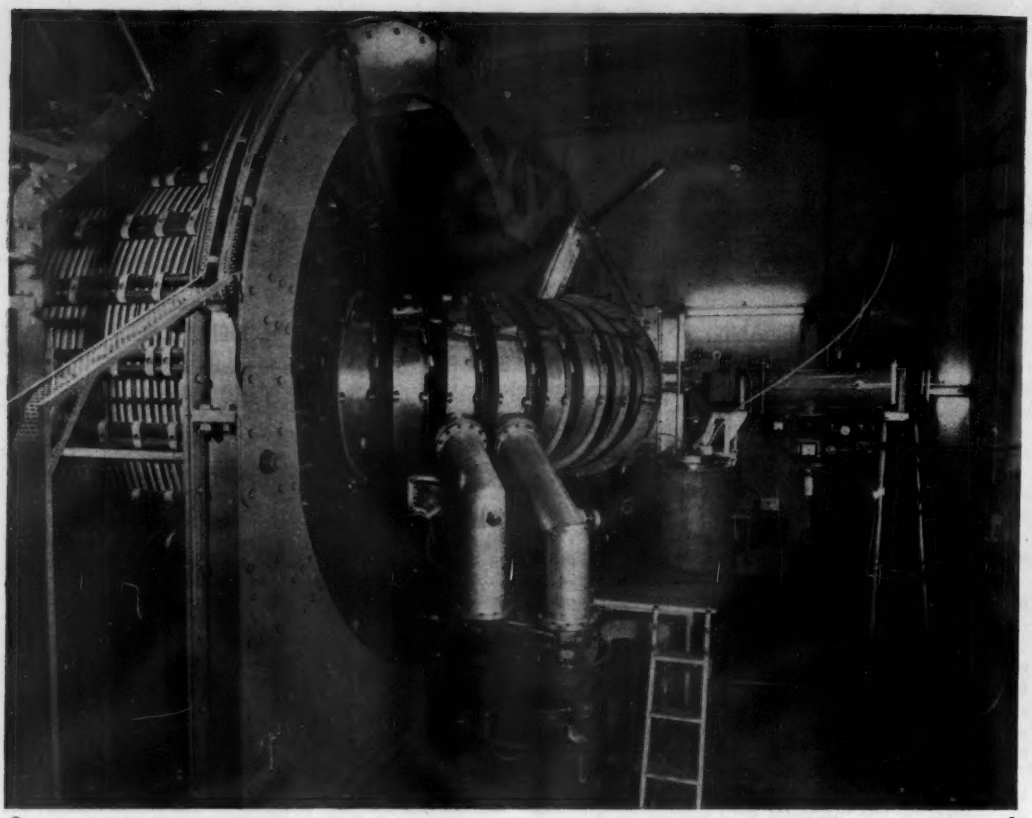


Fig. 1. Photograph of ZETA. The apparatus is enclosed in a room with concrete walls 3 ft. thick for radiation shielding

suppressed by the addition of 5 per cent nitrogen without affecting the neutron yield.

Stability

Measurements with magnetic field-probes and Langmuir probes, together with streak photographs of the current channel taken through a slit in the vacuum vessel, show the current channel to be quasi-stable and clear of the walls for the greater part of the current pulse. Fig. 3 is a reproduction of a streak picture of a helium discharge. The limit of the black area represents the internal diameter of the tube. The light recorded is that of the spark lines of impurities and of (He II) 4686 A. Streak pictures taken of discharges in deuterium are difficult to interpret as the light is emitted by neutral atoms and by impurity ions released into the current channel from the walls.

The centre of the current channel is displaced towards the outer wall due to the tendency of the ring current to expand. This expansion is opposed by eddy currents in the metal walls, which are of 1-in. thick aluminium. Measurements of the internal magnetic fields in the plasma are reproducible and show that the axial magnetic field, B_z, is trapped in the gas. On the axis it increases to approximately ten times the initial value. In general, the resultant lines of magnetic force due to the B_θ and B_z components are helical and vary in pitch over the cross-section of the plasma. The stability of a discharge with this magnetic-field configuration has not been treated theoretically.

The presence of the magnetic field-probe, which is 1 in. in diameter, greatly increases the discharge resistance and reduces the production of neutrons.

The diameter of the current channel estimated from the magnetic-field measurements and the streak photographs is between 20 and 40 cm. at peak current. Transmission measurements with 4-mm. microwaves demonstrate that the electron density

Table 1

Conditions: gas D_2 + 5 per cent N_2. Pressure: $0{\cdot}12 \times 10^{-3}$ mm. B_z = 160 gauss

Current (k.amp.)	Total neutron yield per pulse	T_e (calc.)
84	$0{\cdot}4 \times 10^4$	$2{\cdot}4 \times 10^6$ °K.
117	3·1	2·9
126	9·2	3·3
135	14·2	3·6
141	26·5	3·8
150	41·6	4·0
177	108	4·5
178	125	4·6
187	134	4·65

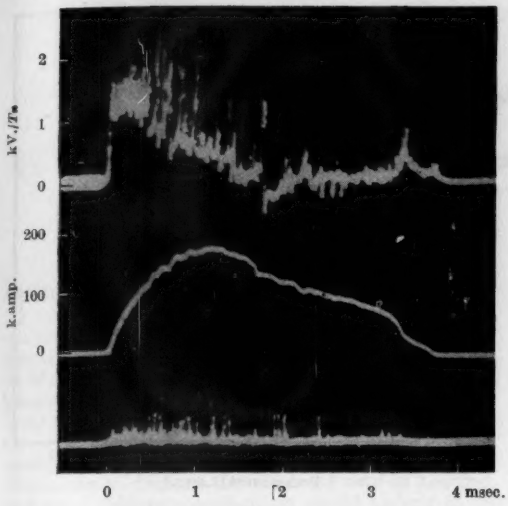


Fig. 2. Oscillograph recordings of the voltage per turn of the transformer, and the secondary current I_s. The lower trace shows the pulses produced by proton recoil in a scintillation neutron counter. Conditions: gas, deuterium + 5 per cent nitrogen + 10 per cent oxygen; pressure, $0{\cdot}13 \times 10^{-3}$ mm. mercury; axial field, 160 gauss

Fig. 3. Streak picture of a helium discharge. Conditions: initial gas pressure, $0{\cdot}25 \times 10^{-3}$ mm. mercury; axial field, 160 gauss; peak current, 130 k.amp. The tube walls lie at the boundary of the dark region

is greater than 6×10^{13} cm.$^{-3}$. This density is consistent with the assumption that all the gas present is ionized and contained in the current channel.

High-Energy Radiations

Neutron emission arising from the D–D reaction is observed for gas currents in deuterium in excess of 84 k.amp. Emission occurs for a period of about 1 msec., centred about the peak current.

Table 1 shows the average number of neutrons emitted per pulse as the peak current is increased.

Fig. 4 is a histogram showing the average rate of neutron emission during the current pulse.

The third column of Table 1 gives the temperatures required to produce the observed neutron yields assuming a thermonuclear process. In calculating these figures, it has been assumed that the current channel is 20 cm. in diameter, emits neutrons uniformly for a period of 1 msec. and all the deuterium initially present is contained in the current channel. Since the reaction-rate is an extremely sensitive function of temperature, variations in these parameters do not greatly affect the calculated temperature T_c. A comparison is made of the calculated and spectroscopically observed temperatures in Fig. 6.

Within the pressure-range investigated ($0{\cdot}8$–$10{\cdot}0 \times 10^{-4}$ mm.) the neutron yield decreased with increasing pressure. Neutrons were observed when 25 per cent of the gas initially present was nitrogen, but the yield was much reduced.

The results obtained with a directional neutron counter moved around the torus showed that, within a factor of two, the neutron emission was uniform and did not arise from localized sources.

No correlation is found between the time of neutron emission and the voltage fluctuations during the current pulse. However, neutrons are produced at the large voltage transient at the end of the pulse, but these can be eliminated by the addition of nitrogen gas.

X-rays are observed towards the beginning of the current pulse. Their average energy lies in the range 20–30 kV. and on the average some 10^6 quanta per pulse are emitted by the whole tube. The number and energy of the X-ray quanta are insensitive to gas pressure and current, but increase in intensity as the axial magnetic field is increased.

Spectroscopic Observations

Both arc and spark line intensities vary greatly over the period of the pulse. In general, emission lines of normal atoms and ions up to three times ionized have a maximum intensity before peak current. Fig. 5 shows the intensity variation of (He II) 4686 A. and (O V) 2781 A.

The Doppler broadening of spark lines emitted in a radial direction is used for estimating the kinetic ion temperature of deuterium and neon discharges. Small quantities of oxygen and nitrogen introduced into deuterium discharges provide spark lines in a convenient part of the spectrum. The breadth of the lines is of the order of 1 A. and can be measured with a quartz spectrograph having a dispersion of 20 A./mm. Calculations show that both Stark and Zeeman effects make a negligible contribution to the line-breadth. Mass motion may contribute to the observed broadening to an appreciable extent, but both probe and streak records show no evidence of

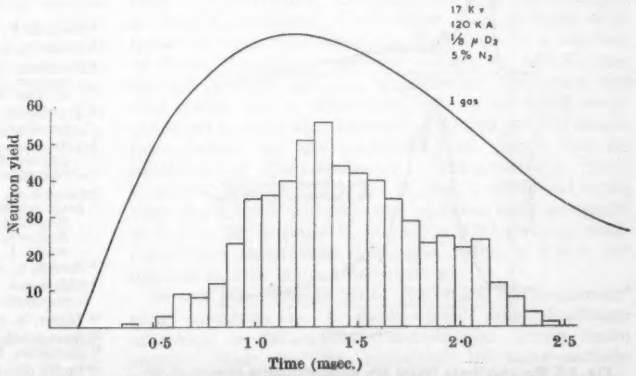


Fig. 4. Histogram showing the number of neutrons counted at various times during the current pulse

gross motion. The contribution to the line breadths of small-scale instabilities and turbulence remains to be measured.

Some 300 emission lines have been identified in the wave-length range 400–2500 A. The most prominent are those of oxygen, nitrogen, aluminium and carbon. Strong lines of (O VI) are recorded. In this wave-length range more than 400 lines remain unidentified.

The (O V) line has been used for Doppler breadth determination without time resolution as it has a maximum intensity in the neighbourhood of peak current. For (N IV) 3479 A. this is not so, and light was admitted to the spectrograph for a period of 1 msec. centred about peak current.

The kinetic temperatures obtained by observation of (O V) and (N IV) lines as a function of peak current are shown in Fig. 6. The ion temperatures are found to decrease with increasing initial pressure of deuterium. No satisfactory measurement of electron temperature has yet been made.

Conclusion

These preliminary results demonstrate that it is possible to produce a stable highly ionized plasma isolated from the walls of a toroidal tube. Hydrogen gas has been maintained in a state of virtually complete ionization with a particle density lying between 10^{13} and 10^{14} per cm.^{3}, for times of milliseconds. The mean energy of the ions in the plasma is certainly of the order of 300 eV., and there are many indications that the electron temperature is of the same order. The containment time and the high electrical conductivity are both adequate for the detailed study of magnetohydrodynamical processes.

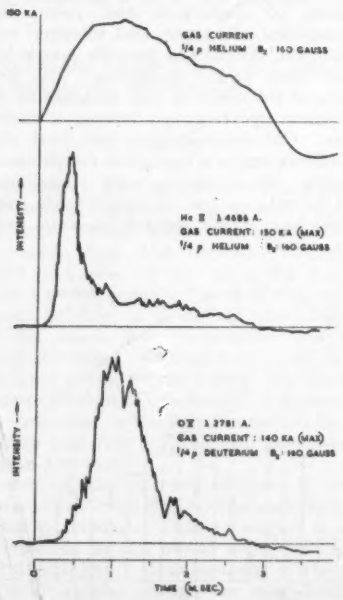


Fig. 5. The two lower traces are photomultiplier records of the intensity variation of two selected spark lines during the current pulse

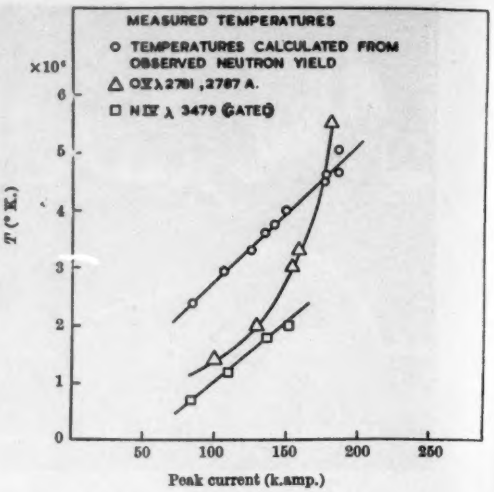


Fig. 6. Ion temperature as a function of peak current determined from the Doppler broadening of (O V) and (N IV). Conditions: initial gas pressure, $0{\cdot}13 \times 10^{-3}$ mm. deuterium and 5 per cent nitrogen; $B_z = 160$ gauss. The temperature of the deuterium gas, estimated from the observed neutron yield, is shown for comparison

To identify a thermonuclear process it is necessary to show that random collisions in the gas between deuterium ions are responsible for the nuclear reactions. In principle, this can be done by calculating the velocity distribution of the reacting deuterium ions from an exact determination of both the energy and direction of emission of the neutrons. The neutron flux so far obtained is insufficient to attain the desired accuracy of measurement.

Investigations leading up to the present results have been constantly encouraged and supported by Sir John Cockcroft and the late Lord Cherwell. The theoretical investigations have been directed by Dr. W. B. Thompson, of the Theoretical Physics Division.

A major part of the engineering design and construction of ZETA was done by the Metropolitan-Vickers Electrical Co., Ltd.

[1] Post, R. F., *Rev. Mod. Phys.*, **28**, 338 (1956).
[2] Pease, R. S., *Proc. Phys. Soc.*, B, **70**, 11 (1957).
[3] Burkhart, L. C., Dunaway, R. E., Mather, J. W., Phillips, J. A., Sawyer, G. A., Stratton, T. F., Stovall, E. J., and Tuck, J. L., *J. App. Phys.*, **28**, 519 (1957).
[4] Kurchatov, I. V., *Atomnaya Energiya*, **1**, No. 3 65 (1956). English translation, *J. Nuclear Energy*, **4**, 193 (1957).
[5] Artsimovitch, L. A., Andrianov, A. M., Bazilevskaya, O. A., Prokhorov, YU. G., and Filippov, N. V. *Atomnaya Energiya*, **1**, No. 3, 76 (1956). English translation, *J. Nuclear Energy*, **4**, 203 (1957).
[6] Colgate, S.
[7] Berglund, S., Nilsson, R., Ohlin, P., Siegbahn, K., Sundstrom, T., and Svennerstedt, S., *Nuclear Instruments*, **1**, 233 (1957).
[8] Curran, S., and Allen, K. W.
} Proc. 3rd Int. Conf. on Ionization Phenomena in Gases, Venice (1957).
[9] Shrafranov, V. D., *Atomnaya Energiya*, **1**, No. 5, 38 (1956). English translation, *J. Nuclear Energy*, **5**, 86 (1957).
[10] Tayler, R. J., *Proc. Phys. Soc.*, B, **70**, 1049 (1957).
[11] Rosenbluth, M.
[12] Bickerton, R. J.
} Proc. 3rd Int. Conf. on Ionization Phenomena in Gases, Venice (1957).
[13] Bezba chenko, A. L., Golovin, I. N., Ivanov, D. P., Kirillov, V. D., Yavlinsky, N. A., *Atomnaya Energiya*, **1**, No. 5, 26 (1956). English translation, *J. Nuclear Energy*, **5**, 71 (1957).

CO-OPERATIVE PHENOMENA IN HOT PLASMAS

By Prof. LYMAN SPITZER, jun.
Princeton University

THE interesting results described by Thonemann and his co-workers in the preceding article provide not only an important step forward in the controlled release of thermonuclear energy, but also a challenging problem in the dynamics of fully ionized gases or plasmas. The spectroscopic line profiles and the neutron counts provide incontrovertible evidence for the acceleration of the positive ions. However, theory would seem to indicate that electron–ion collisions are inadequate to explain the observed rate of heating, and some unknown mechanism would appear to be involved.

The rate at which the positive-ion temperature increases, as a result of electron–ion collisions, has been given elsewhere[1]. It has been suggested, both in Great Britain and in the United States, that this process might be inadequate to explain the rate at which positive ions gain energy in certain electrical discharges. A relatively simple procedure, due to Stix[2], may be used to set an upper limit to the rate at which positive ions are heated by electron–ion collisions. In this method the rate of heating is made a maximum by setting the electron temperature equal to three times the ion temperature, T_i, and setting the electron density equal to a constant, n_e, corresponding to complete ionization and concentration within the discharge channel of all the gas initially present in the tube. On this basis (with $\ln\Lambda$ set equal to 15, see ref. 1) T_i is given by the relation

$$T_i^{3/2} = 1{\cdot}71 \times 10^{-2}\, n_e t \qquad (1)$$

where T_i is in degrees K., n_e in electrons per cm.^{3}, and t in seconds.

To determine the density in the ZETA experiments, we compute the radius, r_d, of the discharge channel on the assumption that the flux of the axial magnetic field, B_z, is held constant in the gas during the contraction, and that the field, B_θ, due to the current equals the compressed axial field at the boundary of the discharge. As a result of the high temperatures achieved in ZETA, appreciable leakage of axial flux out of the discharge does not appear to be possible. Neglect of the finite gas pressure decreases the computed channel radius, increases n_e and again makes the rate of increase of T_i a maximum. On these assumptions, values of r_d have been computed for two currents, and an initial B_z of 160 gauss, and are listed in the second column of Table 1.

Table 1

I (amp.)	r_d(cm.)	n_e (cm.$^{-3}$)	T_i (°K.)	t(theor.)	t(obs.)
126,000	16	$8{\cdot}6 \times 10^{13}$	$3{\cdot}3 \times 10^8$	$4{\cdot}0 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$
140,000	14	$1{\cdot}1 \times 10^{14}$	$2{\cdot}5 \times 10^8$	$2{\cdot}0 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$

These values of r_d are consistent with those cited by the Harwell group. The values of n_e in the third column correspond to complete ionization of all the deuterium initially present in the torus, at a pressure of 1/8 μ, and its concentration in the current channel. The value of T_i for the lower current in column 4 is taken from Table 1 in the article by Thonemann *et al.*, and is based on the neutron yield. The value of T_i at the higher current is the temperature obtained from the Doppler width of the O V triplet, as shown in Fig. 6 of the Harwell article. The ion temperature obtained from the neutron yield under this condition is about 50 per cent greater, but no information is available on the time at which the neutrons appear in this case. In the fifth column are given the values of the time, in seconds, required for the positive ions to reach this temperature, computed from equation 1.

For comparison, the final column lists the observed times, in seconds, at which the positive-ion energies reach the values corresponding to the fourth column. For the lesser current, this is the time at which the neutron yield reaches half its peak value as shown in Fig. 4 of the paper from Harwell. For the greater value of current, t(obs.) is set equal to the time at which the O V radiation reaches its peak intensity, as shown in Fig. 5 of the Harwell paper. The simplifications made have tended to reduce t(theor.), and the correct value may be about an order of magnitude greater than given in Table 1. Thus the discrepancy seems real. There does not appear to be any simple model, based on a quiescent plasma, which is consistent with the observed rapid heating of the positive ions.

Following a suggestion by the Harwell group as to the importance of non-thermal heating processes, Stix[2] in 1956 arrived at conclusions similar to the above from experimental results obtained at Project Matterhorn. A discharge was produced in helium gas in a stainless-steel race-track tube of 10 cm. diameter and 240 cm. axial length, with an initial pressure of 0·63μ and an externally produced axial field of 19,000 gauss. The magnetic field was arranged so that intersection of the outer lines of force with material walls restricted the discharge to a channel of 5 cm. diameter. A loop voltage of 300 V. was applied around an iron transformer threading the race-track, and a maximum current of 8,000 amp. observed; since this current produces only a minor perturbation in the magnetic field, there was no pinching of the discharge. Time-resolved spectroscopic profiles of the He II line, λ 4686, indicated that the kinetic temperature of these ions increased to $1{\cdot}2 \times 10^6$ degrees K. in $1{\cdot}5 \times 10^{-4}$ sec., as compared to a theoretical maximum value of $0{\cdot}8 \times 10^6$ degrees in this same time-interval.

It has been known since the work by Langmuir[3] that electrons in a conventional gas discharge approach a Maxwellian distribution much more rapidly than can be explained by inter-particle collisions. Recent research by Gabor and his collaborators[4] has shown that oscillations generated in

the plasma sheath are responsible for much of this effect, but the detailed mechanism is still unexplained. Possibly the high ion energies observed in ZETA represent a phenomenon related to Langmuir's paradox. To analyse the possible processes involved, such as oscillations, shocks, hydromagnetic turbulences, etc., it would be helpful to obtain information on the extent to which the positive-ion velocities are thermalized, that is, on how nearly the distribution function is isotropic and Maxwellian. Evidently detailed experimental investigations of these co-operative effects in hot plasmas will be of great interest in basic physics.

[1] Spitzer, L., "Physics of Fully Ionized Gases" (Interscience Publishers, 1956, Section 5.3).
[2] Stix, T., Talk at Berkeley, California (February 20–23, 1957).
[3] Langmuir, I., *Phys. Rev.*, **26**, 585 (1925); *Z. Phys.*, **46**, 271 (1928).
[4] Gabor, D., Ash, E. A., and Dracott, D., *Nature*, **176**, 916 (1955).

A STABILIZED HIGH-CURRENT TOROIDAL DISCHARGE PRODUCING HIGH TEMPERATURES

By Dr. N. L. ALLEN, Dr. T. E. ALLIBONE, F.R.S., D. R. CHICK, R. F. HEMMINGS, T. P. HUGHES, Dr. S. KAUFMAN, B. S. LILEY, J. G. MACK, Dr. H. T. MILES, Dr. R. M. PAYNE, J. E. READ, Dr. A. A. WARE, J. A. WESSON and R. V. WILLIAMS

Research Laboratory, Associated Electrical Industries, Ltd., Aldermaston Court, Aldermaston, Berks

SUCCESS in stabilizing a pinched toroidal discharge was first achieved in the apparatus known as ZETA at the Atomic Energy Research Establishment[1]. The stabilization was produced by applying a toroidal magnetic field (B_z) to a gas discharge combined with a metal-walled tube. A discharge similarly stabilized has been obtained in this Laboratory in a smaller apparatus, known as SCEPTRE III.

The discharge tube of SCEPTRE III was an aluminium torus having an internal diameter of 30 cm. and a mean torus diameter of 115 cm. A 4-ton iron core linked the torus with a primary winding of eight turns, wound close to the torus to keep the leakage inductance to a minimum. A condenser bank of 150 μF. charged to voltages up to 30 kV. was discharged through the primary by means of a spark-gap switch. A toroidal magnetic field of up to 1,000 gauss was produced within the torus.

Fig. 1 shows a plot of the peak gas current against applied magnetic field for discharges in deuterium at $3{\cdot}8 \times 10^{-4}$ mm. mercury pressure, the range of currents measured being from 20 to 200 k.amp. Typical

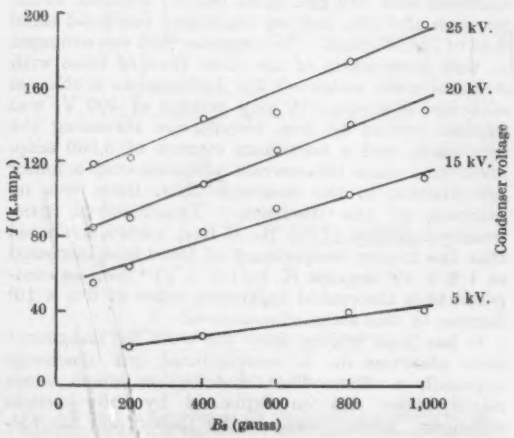


Fig. 1. Current/magnetic field; deuterium, $3{\cdot}8 \times 10^{-4}$ mm. mercury

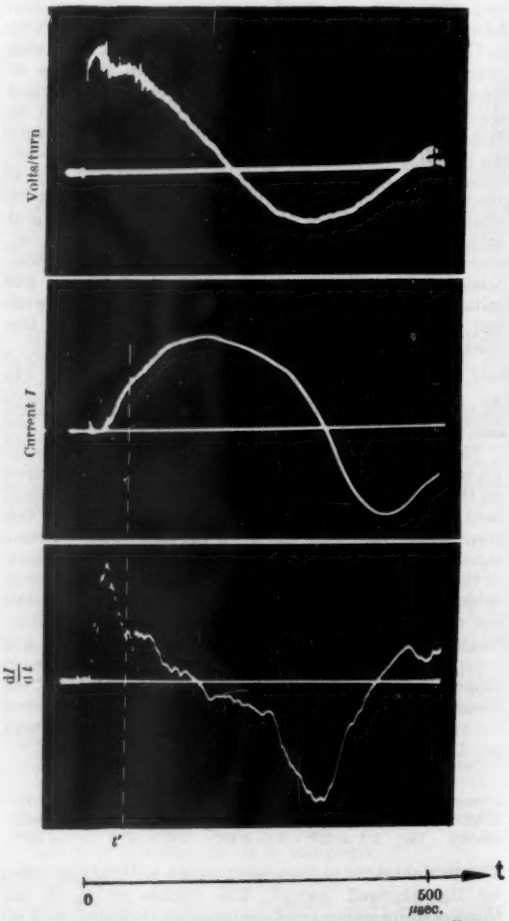


Fig. 2. Deuterium, $3{\cdot}8 \times 10^{-4}$ mm. mercury; condenser voltage, 20 kV.; turns ratio, 8 : 1; magnetic field, 1,000 gauss

Table 1

Pressure (10^{-3} mm. mercury)	Condenser voltage (kV.)	No. of primary turns	B_z (gauss)	Total neutron yield per pulse	Temperature from neutron yield (10^6 °K.)	Temperature from Doppler broadening (10^6 °K.)	Theoretical temperature (10^6 °K.)
1·9	15	16	300	8×10^3	2·7		2·3
1·9	20	16	500	5×10^4	3·3		2·9
1·9	25	16	500	10^5	3·5		3·4
0·9	20	16	200	—		3·2	3·9
3·7	20	16	200	$1·2 \times 10^3$	2·0	2·5	2·2
1·2	25	16	300	—	—	3·8	4·1
0·5	11	8	300	—	—	2·6	2·3

oscillograms of the voltage (V) measured around the torus, the gas current (I) and rate of change of current (dI/dt) are shown in Fig. 2. The marked change of (dI/dt) at t' occurs at that current for which the magnetic field at the wall (B_θ) due to the gas current is equal to the applied B_z field. Theory shows that when these fields are equal, the discharge leaves the walls and constricts, and a sudden change of effective inductance will occur because energy must be supplied to compress the gas and the B_z flux. The change of (dI/dt) at this point confirms this theory. A corresponding change occurs at the same current value when I is decreasing. This is consistent with an expanding discharge reaching the wall at this time. Measurements of inductance at peak gas current derived from the ratio $\left(\frac{dV}{dt}\Big/\frac{d^2I}{dt^2}\right)$ indicate that between 0·55 and 0·9 of the applied B_z flux is trapped by the constricting discharge.

Spectroscopic observations show the presence of a considerable amount of impurity in the discharge (oxygen, carbon, copper and aluminium). These impurities are thought to be produced by the arcing which occurs between the gaps which must be left in the metal walls of the torus. Ion temperatures have been measured by observing the Doppler broadening of the quadruply ionized oxygen lines by means of a Hilger medium quartz spectrograph. The line profiles obtained after allowing for instrumental broadening (by Voigt profiles) were found to be Gaussian down to 1/20 of their peak intensity: this is the experimental limit of observation. At 6×10^{-4} mm. mercury pressure, 15 kV. condenser voltage, and B_z 300 gauss, the line-breadth for the (O V) lines at 2781 A. was 0·97 ± 0·1 A., which with an instrumental width of 0·3 A. corresponds to a temperature of $2·6 \times 10^6$ degree K. No temperature measurements have been derived when operating at less than 11 kV. condenser voltage because of the faintness of the impurity lines. Above this voltage the temperature has been found to decrease somewhat with increased voltage. This disappointing result must be due to radiation and other losses from the discharge increasing more than proportionately with voltage, caused by increased impurity; more arcing does occur at higher voltages.

To reduce the amount of impurity in the gas, experiments were carried out with a greater number of primary turns and hence a lower gradient for a given input energy. Since the impedance of the discharge channel is still predominantly inductive, the peak currents for a given input energy are only slightly reduced. Preliminary measurements with a 16-turn primary have yielded ion temperatures in the range $2·5 \times 10^6$ to $3·8 \times 10^6$ °K., the temperature increasing with condenser voltage up to 25 kV. With deuterium in the torus, neutron emission has been detected over the range of pressures 9×10^{-4} to 7×10^{-3} mm. mercury, a maximum occurring at about 2×10^{-3} mm. mercury, when the magnetic field is about 500 gauss. Table 1 gives some preliminary results for the total number of neutrons per pulse, and in some of the cases the corresponding measured ion temperatures. The temperatures in column 6 of Table 1 are calculated from the neutron yield, assuming a constant density within a discharge channel one-third the diameter of the tube and having a constant temperature for a period of 400 μsec.

Because of the impurities in the discharge, it emits sufficient light to be photographed. Fig. 3 shows a streak photograph of the discharge. The discharge is viewed at one point of the torus simultaneously through a slit window on top of the tube and on the side of the tube. Fig. 3*a* shows a print from a colour photograph which records both red and blue light,

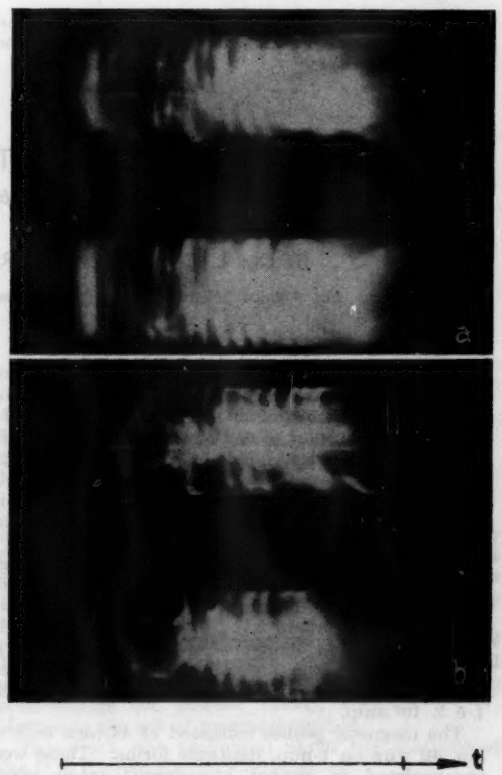


Fig. 3. Streak photograph of the discharge: (*a*) without filter; (*b*) with blue filter. Deuterium, $1·2 \times 10^{-3}$ mm. mercury; current, 55 k.amp.; turns ratio, 8:1; magnetic field, 320 gauss

and Fig. 3*b* a print taken through a blue filter. The main discharge channel is blue and sharp-edged; its diameter decreases as the current increases and becomes unstable after about 300 μsec. when a/b reaches about 0·5, where a and b are the discharge and internal tube radii. The applied B_z was too small in this case to maintain stability throughout the whole pulse time. Superimposed on this channel are bars of light stretching across the whole tube which are mainly red at first, but later become white. These bars have been shown to travel around the torus with velocities of the order 2×10^6 cm. sec.$^{-1}$, and are believed to be due to shock waves produced by the intermittent arcing occurring at the gaps.

The observed results are consistent with the discharge being stabilized, due to the applied toroidal magnetic field being trapped by the constricting discharge. The theoretical conditions for such stability have been studied by Shrafranov[2], Tayler[3] and Rosenbluth[4]. Further properties of a discharge stabilized in this manner have been studied theoretically by Liley, of this Laboratory, in work not yet published. In particular, if the toroidal discharge is produced by discharging a condenser bank through a primary closely coupled with the gas current, the temperature reached at peak current is relatively independent of a/b within the range $1 > \frac{a}{b} > \frac{1}{3}$ and is given in degrees K. by:

$$T = 3 \times 10^7 \times J^{8/5}/(\gamma p b^4 E_0)^{2/5}$$

where J is the energy stored in the condensers in joules per cm. of the discharge tube-length, p the initial pressure of deuterium in microns, and E_0 the initial applied electric field in volts cm.$^{-1}$, γ is a factor near unity associated with skin effect, and b is in cm. This result has been obtained assuming that the gas kinetic pressure is small compared with the magnetic pressure, that the radial heat conductivity is sufficient to maintain uniform temperature across the discharge channel, and that the energy exchange time between positive ions and electrons is small compared with the period of the condenser discharge. Ohmic heating due to currents in both the z and θ directions is taken into account, as is also the effect of adiabatic compression as the discharge contracts. Radiation and other heat losses are neglected.

The temperatures calculated from the above formula are given in column 8 of Table 1; considering the simplifying assumptions which were made, these values agree reasonably well with the experimental observations.

We wish to thank Sir George Thomson for his advice and encouragement throughout this work, and to acknowledge the invaluable assistance received from colleagues at the Associated Electrical Industries, and in particular Mr. J. N. Barnett and Dr. S. E. Hunt; and the stimulus and help resulting from close collaboration with our fellow scientists at the Atomic Energy Research Establishment, Harwell.

[1] Thonemann, P. C., *et al.*, *Nature* (see p. 217 of this issue).
[2] Shrafranov, V. D., *J. Nuclear Energy*, **5**, 86 (1957).
[3] Tayler, R. J., A.E.R.E. Report *T./R.* 2262 (1957).
[4] Rosenbluth, M. N., Third Congress on Ionization Phenomena in Gases, Venice, 903 (1957).

MAGNETIC MEASUREMENT OF PLASMA CONFINEMENT IN A PARTIALLY STABILIZED LINEAR PINCH

By Drs. L. C. BURKHARDT, R. H. LOVBERG and J. A. PHILLIPS
Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

THE distribution of currents in a linear pinched discharge in deuterium has been measured by the use of small magnetic probes inserted into the plasma[1]. This report of work performed in mid-1956 describes the current distribution in a discharge which compresses a longitudinal magnetic field originally set up in the tube by an external coil. The stabilizing properties of an included longitudinal field have been discussed theoretically elsewhere.

The discharge tube was of the Columbus[2] design with an inside diameter of 6·8 cm. and an electrode spacing of 30 cm. Energy was stored in a capacitor bank of 75 μF. at 10 kV. The connecting circuit, consisting of ten ignitrons and coaxial cables in parallel, had an inductance of 0·12 μH., which allowed a peak current in the discharge of approximately $1{\cdot}5 \times 10^5$ amp.

The magnetic probes consisted of 44-turn coils of No. 40 wire on 1-mm. diameter forms. These were encapsulated in slotted hypodermic tubes which provided electrostatic shielding, and these in turn were surrounded by quartz jackets for insulation from the plasma. The coil output was integrated by

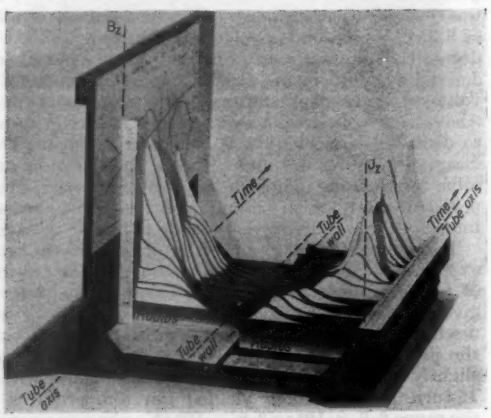


Fig. 1. B_z magnetic field and axial current density (J_z) distributions as a function of time. Initial conditions: B_z = 1,000 gauss; deuterium pressure, 600μ; V, 10 kV.

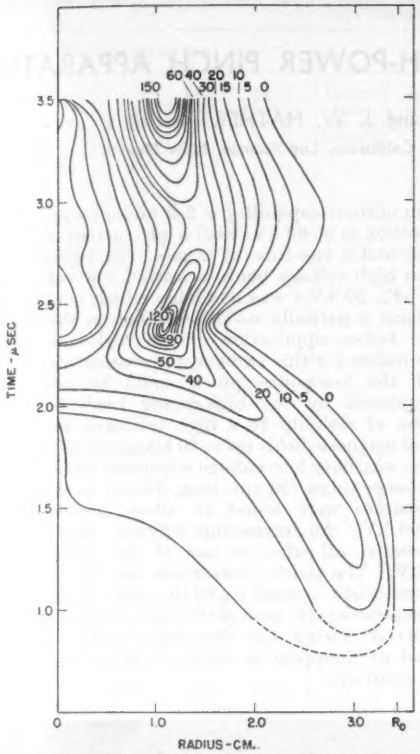


Fig. 2. Contour plot of axial J_z current density as a function of time. Same conditions as in Fig. 1

an RC network and displayed on an oscilloscope the sweep of which was triggered by the initial derivative of discharge current.

Plots of the B_θ and B_z fields were made by firing the tube and photographing the traces from the appropriately oriented probe at each of a family of equally spaced radial intervals. These traces of $B(t)$ for different radii were used for constructing curves of $B(r)$ for separate times. Differentiation of these traces then yielded distributions for j_θ and j_z. The requirement for good reproducibility of traces from shot to shot was well met up to approximately three microseconds, when the discharge, not completely stabilized by the 1,000-gauss B_z, went into a kink instability and became turbulent, filling the tube.

The experimental results for a discharge in deuterium at 600μ with 1,000-gauss B_z applied initially are shown in Figs. 1, 2 and 3. The discharge is seen to be compressed to approximately one third the tube radius and to oscillate about a mean value. The B_z field is compressed within the main current sheath.

If B_z and B_θ are known throughout the tube, and if one assumes the pinch to be in an equilibrium state, the plasma pressure may be calculated from the equation: $\mathbf{j} \times \mathbf{B} = \nabla p$.

In this case, the pinch was not entirely in equilibrium, as evidenced by a tendency to 'bounce' slightly at maximum compression. However, pressure balance calculations were made at times when the sheath acceleration was zero, where the above equation also applies.

Fig. 4 shows a plot of $B_z(r)$, $B_\theta(r)$ and $p(r)$ at 2·5 μsec. after firing the discharge. The pressure

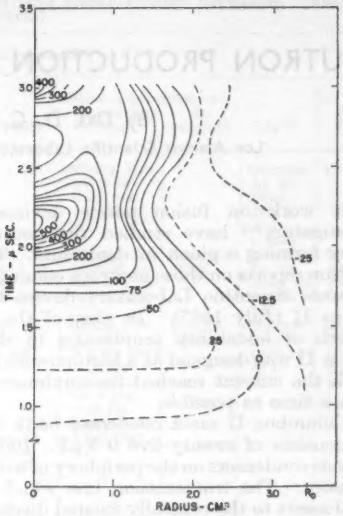


Fig. 3. Contour plot of B_z magnetic field as a function of time. Same conditions as in Fig. 1

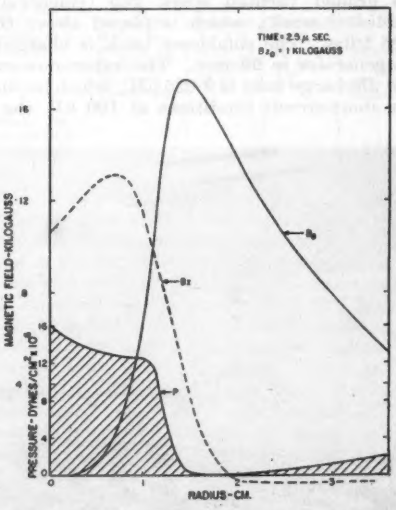


Fig. 4. B_θ and B_z magnetic fields plotted as a function of radius at 2·5 μsec. under conditions of Fig. 1. The radial material pressure as calculated from pressure balance considerations is also shown

corresponds to a kinetic temperature of approximately 10 eV. if all the gas is assumed to have been swept in with the current sheath. In later stages in the discharge the maximum temperature before breakup was about 40 eV.

We wish to acknowledge the encouragement of J. L. Tuck during this work. L. Fretwell assisted generously in the taking of results. We also acknowledge the pioneer (1954) work of J. W. Mather in the use of magnetic probes.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

[1] Kurchatov, I. V., Moscow 1956, reported at Harwell, May 1956.
[2] Burkhardt, L. C., Dunaway, R. E., Mather, J. W., Phillips, J. A., Sawyer, G. A., Stratton, T. F., Stovall, E. J., and Tuck, J. L., *J. App. Phys.*, 28, 519 (1957).

NEUTRON PRODUCTION IN A HIGH-POWER PINCH APPARATUS

By Drs. D. C. HAGERMAN and J. W. MATHER
Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

IN the work on fusion power devices, several investigators[1-5] have studied the neutrons produced by forming a pinch in deuterium. This communication reports on those neutrons coming from the Los Alamos Scientific Laboratory device known as Columbus II (July 1957). In view of the fast rate of growth of instability amplitudes in the pinch, Columbus II was designed as a high-powered machine in which the current reached its maximum value in as short a time as possible.

The Columbus II main condenser bank (shown in Fig. 1) consists of twenty-five 0·8 μF., 100-kV. low-inductance condensers on the periphery of a circle 14 ft. in diameter. The transmission line which connects the condensers to the centrally located discharge tube consists of two circular copper sheets separated by a $\frac{1}{4}$ in. polyethylene insulator. This bank is switched by a graded vacuum spark gap (Hagerman, D., unpublished work), which is placed above the discharge tube. The condenser bank is charged by a Marx generator in 90 μsec. The inductance external to the discharge tube is 0·035 μH., which implies that under short-circuit conditions at 100 kV. the maximum current capability is 2·5 million amp. Typical operation is at 60 kV. with a gas current of 1 million amp. and a rise-time of 2 μsec. In addition to the main high-voltage bank, a second low-voltage bank (60 μF., 20 kV.) was available which could be used to form a partially stabilized pinch in the discharge tube before application of the high-voltage bank. The reason for this two-stage operation was the hope that the low-power pinch could be adiabatically compressed by the high-power bank beyond the region of stability to a high transient temperature. Axial magnetic fields up to 10 kilogauss were provided by an auxiliary low-voltage condenser bank. Ceramic discharge tubes (30 cm. long, 10 cm. in diameter) of sillimanite were found to allow operation up to 55–60 kV. An interesting difficulty has up to date prevented all effective use of the machine above 60 kV. The pinch phenomena can be identified by characteristic voltage or, in the case of extremely low inductance supply such as this one, current fluctuations observed during the discharge cycle. These are found to disappear at voltages above 55 kV. as do the neutrons.

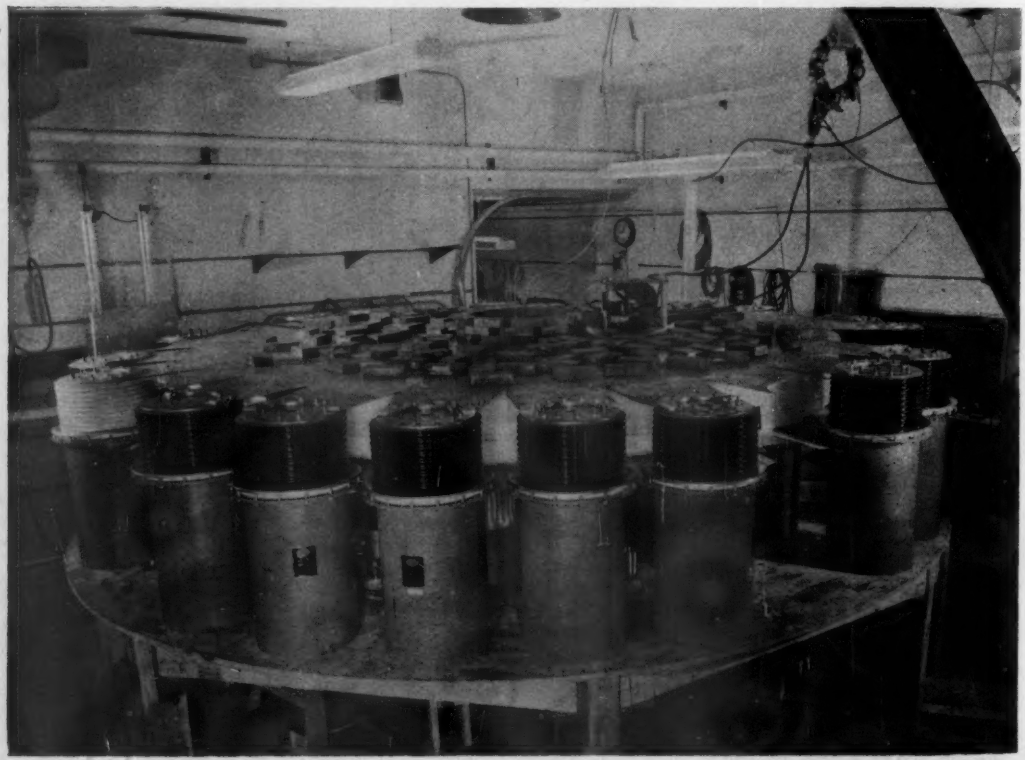


Fig. 1. General view of the apparatus

Table 1. COMPILATION OF TYPICAL DATA CHARACTERIZING NEUTRON OBSERVATIONS FROM LINEAR PINCHED DISCHARGES. (UNREPORTED DATA REPRESENTED BY BLANK SPACES)

	Electrical parameters			Neutron parameters								
								Neutron dependence on:				
Reference	V_0 (kV.)	$I_{max.}$ (k.amp.)	Rise-time (μsec.)	Pulse duration (μsec.)	Occurrence No. of bounces	Max. yield per pulse	Anisotropy in neutron energy (MeV.)	B_z field	Contamination	D_2 pressure	I or V	Neutron distribution
a 1	40	400	~8	~1·0	2	4×10^7			Strong. Vanish with 0·01 per cent H_2, A, Xe		Increase by 20 from 220 to 450 k.amp.	Uniform
b 2	35	~300	2·0	~0·3	2, 3, 4	10^7–10^8	~0·3	Strong. Decreased by factor 50 for 50 gauss	Strong. Decreased by factor of 50 for 8μ of A.	Peak at 200μ	Increase by 20 from 15 to 30 kV.	Uniform axial plates
c 3	15	250	5·0	0·1	2, 3	10^7–10^8	~0·26	Strong. Vanish with 100 gauss	Strong. Vanish with 0·2 per cent N_2. Decrease to $\frac{1}{2}$ for 5 per cent He	Peak at 125μ	Increase by 50 from 14 to 17 kV.	Linear increase from anode to cathode
d 4	10	300	5·0	~0·5	3	10^8		Weak. Decrease by factor 10 for 1,000 gauss	Vanish with 5 per cent N_2			
e 5 Columbus II	23	200	6½	~1·0	1, 2, 3							
f Preheaters alone	15	250	6	~0·1–0·3	1, 2	2×10^7		Strong. Decrease by factor 6 for 23 gauss				
g Main bank alone	50	800	2·0	~1·5	1	3×10^8	0·17	Weak. Decrease to $\frac{1}{2}$ for 125 gauss	Weak. Decrease according to $(1-f)^2$ for He up to 50 per cent	Increase by 2·3 from 70 to 200μ of D_2	Increase by 2 from 40 to 50 kV.	Non-linear 0–5 cm. at anode. Uniform 5 cm. to cathode
h Preheater and main bank	50	~800	2·0	~1·0	1	7×10^7		Weak. Decrease to $\frac{1}{2}$ for 90 gauss				

(*a*) *Low-voltage* (*preheater*) *bank alone.* Neutrons were produced in short bursts (0·1–0·3 μsec. long) occurring approximately at the time of the first and second contraction of the pinch. The maximum yield of neutrons was 2×10^7; the yield decreased by a factor of 6 by the addition of a 23-gauss axial magnetic field, which suggests that these neutrons may be produced by some instability mechanism. (One form of instability mechanism is discussed in ref. 3.)

(*b*) *High-voltage bank alone.* Fig. 2 shows di/dt and i for 50-kV. operation. The neutrons appear shortly after the first contraction, as indicated by the small dip in the current. Fig. 3 shows typical neutron pulses with and without an axial (B_z) magnetic field. With no B_z field a short (0·3 μsec.) neutron burst with little or no tail was observed. A small B_z field lowers the relative height of the initial burst of neutrons and enhances the number of neutrons appearing in the tail of the neutron pulse. Neutron-pulse durations of up to 1·5 μsec. were observed with B_z fields as small as 200 gauss. The total yield of neutrons was rather insensitive to the axial magnetic field, dropping by only 55 per cent when the field was changed from 0 to 125 gauss. The maximum yield observed for zero B_z field was 3×10^8 neutrons. The dependence of the neutron yield on pressure and helium contamination showed: (1) that the yield was proportional to the pressure, increasing by a factor of 2·3 between 70 and 200μ; (2) the fraction f of the helium contamination lowered the yield according to the expression $(1-f)^2$ (these results were obtained with a B_z field of 200 gauss). Fig. 4 shows the axial neutron energy distribution from nuclear

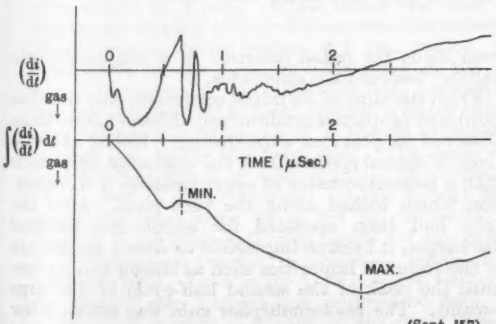


Fig. 2. Gas current and $(di/dt)_{gas}$ vs. time

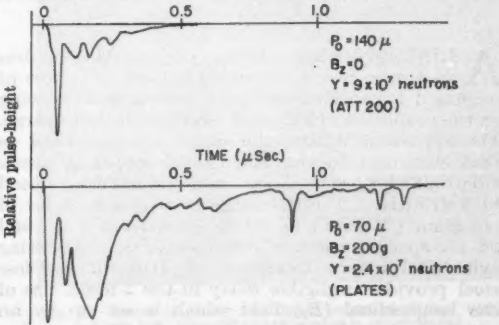


Fig. 3. Typical neutron pulse shapes

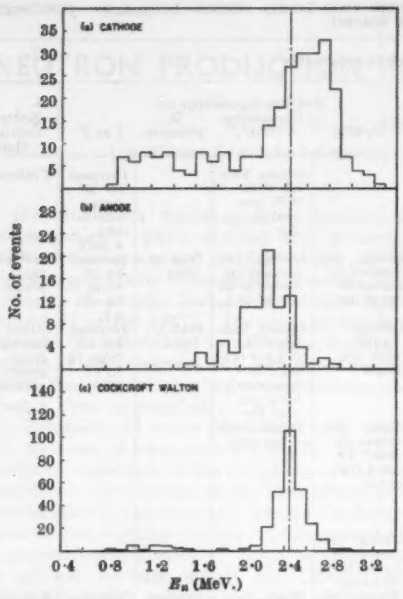


Fig. 4. (*a*) and (*b*) show the axial neutron energy distribution; (*c*) a range-energy calibration using a Cockcroft–Walton machine

plates situated behind the cathode and anode. The observed energy shift of approximately 0·17 MeV. corresponds to a 20-keV. deuteron colliding with a deuteron at rest, or to a group of interacting deuterons travelling toward the cathode with a velocity of 7×10^7 cm./sec. Plates were also exposed radially at three positions along the discharge; the results are compatible with a uniform neutron distribution from the cathode to within 5 cm. of the anode, beyond which the yield is lower. All the plate exposures were made with a 200-gauss B_z field and 50-kV. bank voltage.

(*c*) *Operation of banks in combination.* These results are preliminary. (1) Time-sequence operation was achieved; however, timing had little effect on neutron yield if the fast bank was timed prior to the development of instabilities in the first-stage pinch. (2) Neutron pulse widths were approximately 1 μsec. (3) Neutron yields were approximately 10^7/pulse. (4) A 90-gauss axial magnetic field reduced the yield by a factor of 3. (5) Neutron yield increased by a factor of 2 in going from 40 to 50 kV. on the high-voltage bank. In Table 1 the above results are compared with those reported by other laboratories.

The dependence of neutron yield (row *g*, Table 1) on applied magnetic field, helium contamination, gas pressure and voltage, as well as the long neutron pulse-duration, suggests a mechanism of neutron production different from the usual instability process. However, it is clear from the anisotropy in the neutron energy spectrum at 200-gauss axial magnetic field that directed motion of the reacting deuteron system exists. This anisotropy is smaller than that reported (row *b*, *c*, Table 1) for instability neutrons without axial magnetic field, even though the applied electric field in Columbus II is appreciably larger.

We are indebted to J. L. Tuck and J. A. Phillips for many helpful discussions concerning this experiment. Thanks are also due to L. Rosen and the nuclear plate group for their generous assistance, to J. H. Coon for the provision of D–D calibration neutrons, to L. Neher for assistance in fast-scintillation techniques, and to A. H. Williams and Glen Livermore for their technical assistance throughout.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

[1] Artsimovich, L., Andrianov, Bazilevskaya, Prokhovov and Filippov, *Atomnaya Energiya*, **3**, 76 (1956).
[2] Anderson, D. A., Baker, W. R., Colgate, S., Gilbert, F. C., Ise, J., Pyle, R. V., and White, R. S., Paper at Conference on Plasma Physics, Venice (June 1957).
[3] Dunaway, R. E., and Phillips, J. A., *LADC* 2943 (to be published).
[4] Bodin, H. A. B., and Reynolds, J. A., *Engineering*, **184**, No. 4781, 538.
[5] Berglund, Nilsson, Ohlin, Siegbahn, Sundstrom and Svennerstedt, *Nuclear Instruments*, **1**, 233.

NEW CONFINEMENT PHENOMENA AND NEUTRON PRODUCTION IN A LINEAR STABILIZED PINCH

By Drs. L. C. BURKHARDT and R. H. LOVBERG

Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

A LINEAR discharge device[1], designated Columbus *S*–4, has been constructed to test the effect of increased tube diameter and inter-electrode spacing on the properties of a B_z-stabilized pinched discharge. The apparatus utilizes the same capacitor bank as that employed in the magnetic-probe experiments (75 μF., 20 kV.) with the external inductance reduced to 0·075 μH. The discharge tube is a cylinder of porcelain ('Mullite') of inside diameter 5·0 in. and 24 in. spacing between electrodes. A close-fitting cylindrical return conductor of 1/16 in. stainless steel provides negligible delay in the 2-msec. rise of the longitudinal (B_z) field which is set up by an external solenoid and capacitor bank. Provision has been made for radial insertion of a magnetic probe halfway between the electrodes.

From the time of its initial operation, this tube has exhibited properties qualitatively different from those observed in previous experiments. Initial observations of optical spectra from the discharge were made with a monochromator of approximately 1 A. resolution which looked along the tube axis. After the tube had been operated for about one hundred discharges, it became impossible to detect the spectra of the common impurities such as silicon and oxygen until the peak of the second half-cycle of discharge current. The photomultiplier gain was set an order of magnitude higher than the value adequate for use

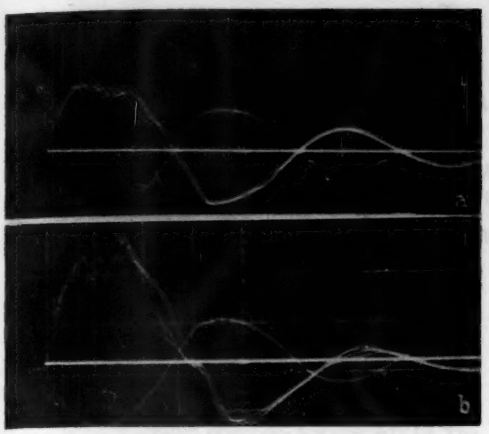


Fig. 1 (*a*) Four superimposed traces of $B_\theta(t)$ for a radius of 6·4 cm. (tube-wall radius). The capacitor bank current is shown in opposite polarity. Sweep : 5 μsec./cm. (*b*) B_θ traces for $r = 4·4$ cm.

on previous systems. The H_β line, under these conditions, appeared with considerable amplitude during the initial discharge current rise, dropped to an almost undetectable amplitude near the first current maximum, and remained faint until the second current maximum, when it again became prominent. The rise in gas pressure after each shot was less than 5 per cent. Operating conditions for the above observations were :

Condenser voltage	$= 1·5 \times 10^4$ V.
B_z	= 1,750 gauss
Deuterium pressure	= 40μ mercury
$I_{max.}$	$= 2·5 \times 10^5$ amp.
Time to first current peak	= 6 μsec.

Magnetic-probe measurements of the B_θ and B_z fields were made under these conditions, and for times extending through three half-cycles of the bank current. While the shot-to-shot reproducibility of the probe traces became imperfect after 3 μsec., due to an apparent small-amplitude flutter of the plasma column, the spread in the trace amplitudes was still sufficiently small to allow the plotting of meaningful field distributions and the calculation of approximate plasma pressures. Figs. 1*a* and 1*b* are B_θ records for radii of 6·4 cm. (the tube wall) and 4·4 cm. respectively. Four traces are superimposed in each picture, and the capacitor bank current is also displayed in opposite polarity. The sweep-speed is 5 μsec. per cm. An unexpected effect is seen to manifest itself after a time of 3 μsec. While before this time the amplitudes display the $1/r$ dependence characteristic of all current being within 4·4 cm. radius, this dependence breaks down at later times in the half cycle ; in particular, the amplitude ratio between the two sets of traces at 10 μsec. exceeds a factor of two. This implies the existence of a sheath of negative current just inside the tube wall. The same conclusion can be drawn by comparing the B_θ amplitudes at the zero of input current ; no net current flows inside the tube, since B_θ at 6·4 cm. is zero ; yet about 5×10^4 amp. is flowing inside 4·4 cm. radius. A circulation of current is evidently taking place inside the tube. Later in the cycle it is seen that when the central core current has dropped to zero, the entire bank current is flowing in the outer sheath ; this proceeds to pinch, and the above process is repeated. It is noteworthy that contaminant spectra appear only at the compression of this second sheath ; wall materials have apparently collected in it during its relatively long existence at the wall.

A pressure-balance calculation has been done using the B_z and B_θ amplitudes at 6 μsec. after the starting of current, that is, at the current maximum. The plasma pressure at the tube axis and at this time appears to be about 12 atmospheres ; if the plasma is assumed to be uncontaminated and pinched to the same radius as the longitudinal current, the temperature should be in excess of 300 eV.

Since a temperature as high as this, together with the assumed central density, should result in an easily detectable neutron yield, namely, of the order of 100/c.c.-μsec., a series of counter experiments was performed. A plastic scintillation counter was found to give pulses centred at the current maximum, and tests with paraffin interposed between the counter and discharge tube established the radiation to be neutrons. The yield was found to be too small to activate a silver foil counter to significantly greater than a background count, and it was concluded from the known counter calibration that the total yield was fewer than 10^5 per discharge. The numbers of counts observed in the plastic scintillators made it apparent that some of the discharges yielded more than 10^3 neutrons. At the time of writing, the number has not been accurately fixed. Fig. 2 shows the output of the scintillation counter for several successive discharges, along with a trace of the condenser current. In this instance, the condenser voltage had been raised to 17·5 kV.

Runs were made to measure relative neutron yield as a function of bank voltage, B_z, deuterium pressure, and contaminant fraction in the gas. The yield over the half-cycle was obtained by integrating the photomultiplier anode current for this time. Ten shots were taken at each condition of the system. In Figs. 3, 4, 5 and 6 the relative yield for each discharge is plotted as a small dot, while the mean values are horizontal bars.

It must be remarked that most of the data plotted in the above figures were taken at conditions more favourable for neutron production than those used for the magnetic-field measurements. However, counts were observed in significant numbers at 15-kV. bank voltage and 1,750-gauss B_z ; typically, one observed the order of 10 counts per discharge in a 5 in. × 5 in. cylindrical plastic scintillator placed

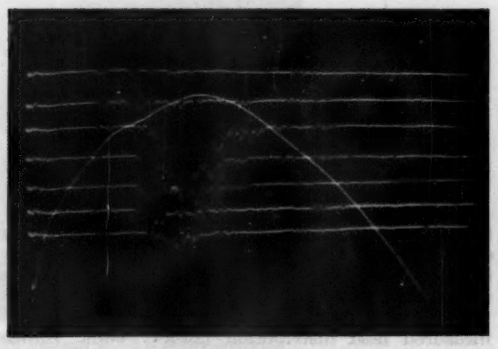


Fig. 2. Traces of plastic scintillator output vs. time ; sweep, 2 μsec./cm. Capacitor bank current is superimposed

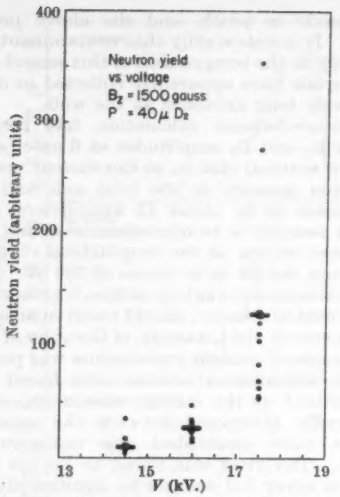


Fig. 3. Neutron yield vs. capacitor bank voltage. The small dots represent yields for individual discharges; ten discharges were measured at each value of V. The bars are mean values for the ten points

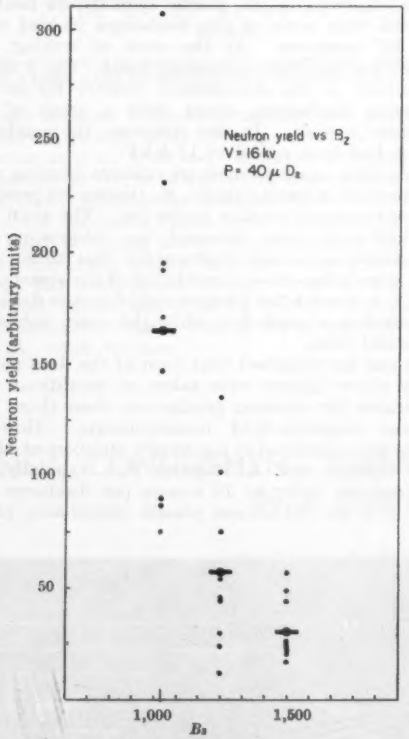


Fig. 4. Neutrons vs. B_z

8 in. from the centre of the tube. At this condition, as in Fig. 2, the neutrons are produced in a time interval 4 μsec. wide, centred near the current maximum.

While the pinch configuration for the case of the measured field distribution (15-kV. bank voltage, 1,750 gauss B_z) is clearly stable against break up by kinking, and the 16 kV., 1,500-gauss condition also

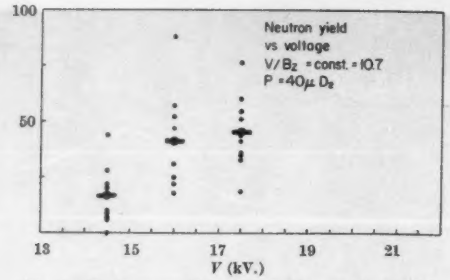


Fig. 5. Neutrons vs. V for V/B_z held constant at 10·7

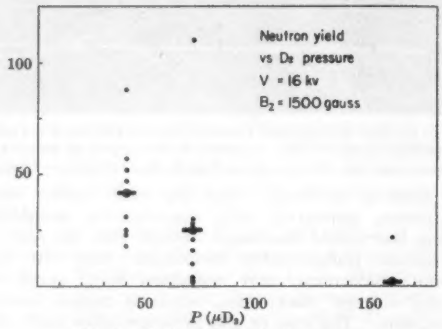


Fig. 6. Neutrons vs. deuterium pressure

appeared stable in this sense from initial probe surveys, it is not established that the runs which involve higher ratios of bank voltage to B_z are stable. One must, therefore, allow the possibility that the neutron output in the lowest B_z and highest bank voltage cases results from some acceleration mechanism associated with instability. Even in the configuration of which B_θ traces are shown, the fluttering of the fields near maximum current may represent strong local field inhomogeneities which could provide a particle-accelerating mechanism. It is clear, however, that the variations of yield with V and B_z are consistent with a thermonuclear origin of the neutrons, since the plasma compression and current density are sensitive to variation of these parameters.

The lower limit of gas pressure was set by the inability of the tube to break down at less than 40μ.

A contaminant of 5 per cent air in the deuterium reduced the neutron output to less than a tenth of its normal value.

Work is progressing on the problem of determining pinch stability for the conditions where it is now uncertain. An effort will also be made to measure the degree of energy isotropy of the neutrons, since anisotropic energy distribution has in the past identified the neutron yield from several devices as being non-thermonuclear[2].

We wish to thank J. L. Tuck and J. A. Phillips for helpful discussions during the course of this work. We also acknowledge the contribution of H. Dreicer in predicting theoretically the entrapment of wall impurities by a plasma sheath.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

[1] Burkhardt, L. C., Dunaway, R. E., Mather, J. W., Phillips, J. A., Sawyer, G. A., Stratton, T. F., Stovall, jun., E. J., and Tuck, J. L., *J. App. Phys.*, **28**, 519 (1957).

[2] Dunaway, R. E., and Phillips, J. A., Los Alamos Report, *LADC* 2943 (to be published).

NEUTRONS FROM A STABILIZED TOROIDAL PINCH

By Drs. J. HONSAKER, H. KARR, J. OSHER, J. A. PHILLIPS and J. L. TUCK

Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

HYDROMAGNETIC calculations on idealized models of the pinch have indicated that stability might be attained by a combination of a central axial magnetic field and conducting walls, provided that certain restrictions on compression and $8\pi p/B^2$ (p is plasma pressure) are obeyed[1–5]. The problem is actually more complicated than these simple models suggest, especially when the finite thickness of the boundary layer between external magnetic confining field B_θ and internal magnetic stabilizing field B_z is taken into account.

Experiments at Los Alamos on straight tubes[6] using magnetic probes have measured substantial plasma pressure confinement with appreciable stability by these means. It proved unexpectedly difficult to do as well in toroidal geometry, due in part to the technical difficulty of inducing high-voltage gradients and also to the presence of unavoidable gradients in electric and magnetic fields across the discharge tube cross-section. After a progression of machines extending over several years, Perhapsatron S–3 began to show (middle 1957), by magnetic probe measurements[7], more symmetrical detachment of the current channel from the walls. This apparatus is shown in Fig. 1.

Fig. 2 shows a magnetic pressure measurement with plasma pressure (nkT) determination made in Perhapsatron S–3 at two stages in the current wave $t = 4$ μsec., $2{\cdot}0 \times 10^5$ amp., and $t = 6$ μsec., $2{\cdot}3 \times 10^5$ amp. Such curves show penetration of the B_θ magnetic field towards the pinch axis with some increased confining current density along the axis and little current outside the pinch. We evaluate nkT by substitution in the pressure balance equation:

$$\left[nkT + \frac{B^2}{8\pi}\right]_{r_1}^{r_2} = -\frac{1}{4\pi}\int_{r_1}^{r_2}\left(\frac{B_\theta^2}{r} + \frac{B_z^2}{r}\right)dr + C$$

where $B^2 = B_\theta^2$, r and R are the radii of curvature of B_θ and B_z respectively, and C is a constant evaluated at some position at which nkT is known. The calculations of nkT are not precise, since (1) the radii of curvature of the magnetic field lines are assumed from symmetry and (2) uncertainties of the position of the discharge axis seriously affect the integration in its vicinity. The pressures are consistent with appreciable average temperatures greater than 125 eV. Also the cooling effect of impurity influx from the walls is suggested by the lower pressures at 6 μsec., compared with those indicated at 4 μsec. This is paralleled by the rise in impurity spectral line inten-

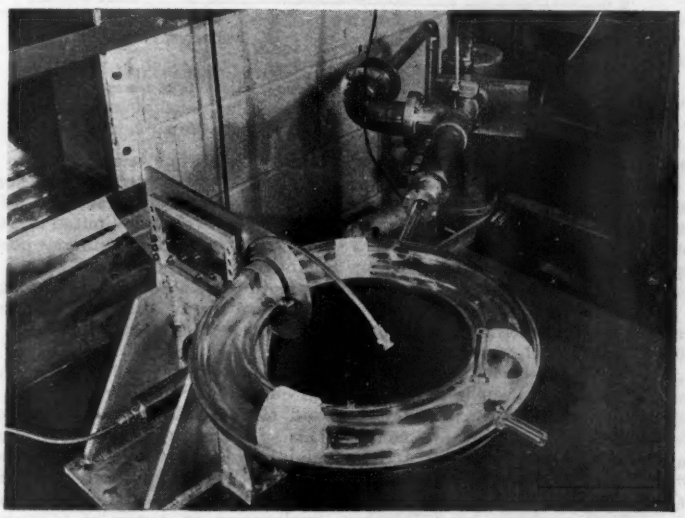


Fig. 1. The glass torus (minor radius 2·65 cm.) of Perhapsatron S-3 is shown supported in the lower half of the copper primary (major radius 16·2 cm.). The condenser is 225 μF. (44,500 joules at 20 kV.) with a total connected inductance of 0·08 μH. at the torus feed-point

sities emitted from the discharge, up to this time quite faint (Fig. 3*f*). It was also observed that the small magnetic probe, diameter 1·7 mm., when extended into the discharge, caused the impurity lines to appear earlier. This suggests that the temperature would be higher and the current sheath thinner when the probe was absent.

A large liquid scintillator (43 cm. diameter, 50 cm. high) showed neutron emission (December 17, 1957) of 10^4 per discharge. Substitution of a quartz ('Vycor') torus for the 'Pyrex' glass one raised the average output to 2×10^5 neutrons per discharge. Addition of a laminated iron core improved the coupling in the early stages of the current cycle before it saturated, and gave a further small increase. Neutron yields up to 10^6 per discharge have been observed.

Neutron intensity versus time as detected by a proton recoil scintillation counter shows a burst of duration approximately 2 μsec. starting at about 3·0 μsec. after gas breakdown, with successive smaller bursts superimposed on a tail of duration 10–15 μsec. (Fig. 3*c*). It seems definite that some neutron emission continues through the discharge current zero and occasionally up to and beyond the second current maximum (~30 μsec.). A pulse of neutrons is at times associated with the second current zero ~60 μsec. This may have interesting implications. There is a well-defined optimum B_z, with no yield at zero B_z (Fig. 4). The yield rises with diminishing deuterium pressure, and the lower limit in this direction is determined by failure to obtain gas breakdown (Fig. 4) even with radio-frequency pre-excitation. The yield is sensitive to contaminants and to the quality of the base vacuum pressure. 5 per cent nitrogen reduces the yield by a factor of 2 (Fig. 5). Yields are variable at fixed operating conditions by a factor of about 3.

X-rays appear in a sharp burst, duration approximately 1 μsec., about 0·9 μsec. after gas breakdown, which is before the time of neutron emission. Little or no X-ray yield is observed under the conditions of maximum neutron yield. The X-ray intensity increases with deuterium pressure, whereas the neutron intensity increases with decreasing pressure. At high pressures (400μ), X-ray energies as high as 0·9 MeV. have been measured with 12 kV. primary voltage and 5,000 gauss B_{z_0}.

A spectrogram taken during three complete discharges at 11 A. per mm. dispersion over range 3000–6600 A. shows a strong continuum throughout, with a few lines greatly broadened ~30 A. tentatively identified as Si II, Si III.

One of the central features of the design of Perhapsatron *S*-3 is the thinness (0·3 cm.) of the glass walls of the torus and its close fit to the metal primary, based on the unequivocal predictions by theory of the bad effect of B_z external to the pinch on stability. This has made the glass torus very fragile and easily broken by strains transmitted to it from the heavy primary under electromagnetic forces. The neutron yield rises between 14 and 15 kV. primary voltage, with higher voltages limited by failure of the glass torus. A precision alumina porcelain torus when delivered is expected to reduce these difficulties, with the possible added advantage that alumina is known from other experiments to be superior to quartz in the contaminants it supplies to the discharge.

A temperature of 600 eV. (compression 9) would be consistent with the observed yield of neutrons, and the pressure balance equations would support this. Magnetic probes indicate that the pinch undulates as a whole with an amplitude of about 2 mm. The diminution in neutron yield after the initial burst is a matter for concern and finds an explanation in terms of cooling by the influx of impurities (~3·6 μsec.). The observed sensitivity to impurities is supporting evidence for a thermonuclear reaction but has been misleading in the past—

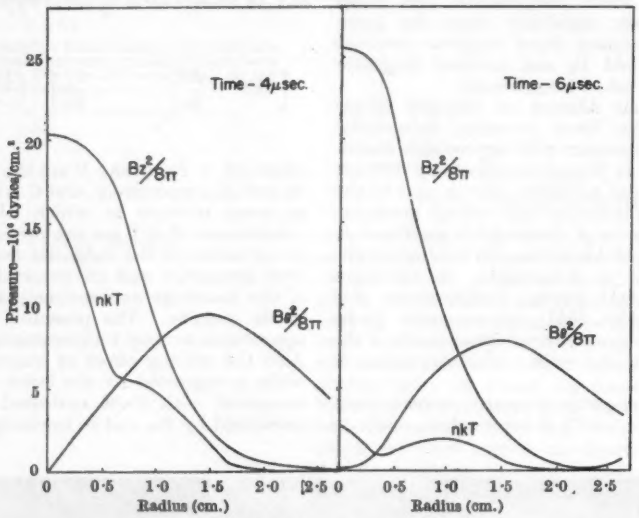


Fig. 2. Magnetic field pressure distributions as a function of radius at $t = 4$ μsec. and 6 μsec. The material pressure (nkT) distributions calculated from pressure balance considerations are also shown

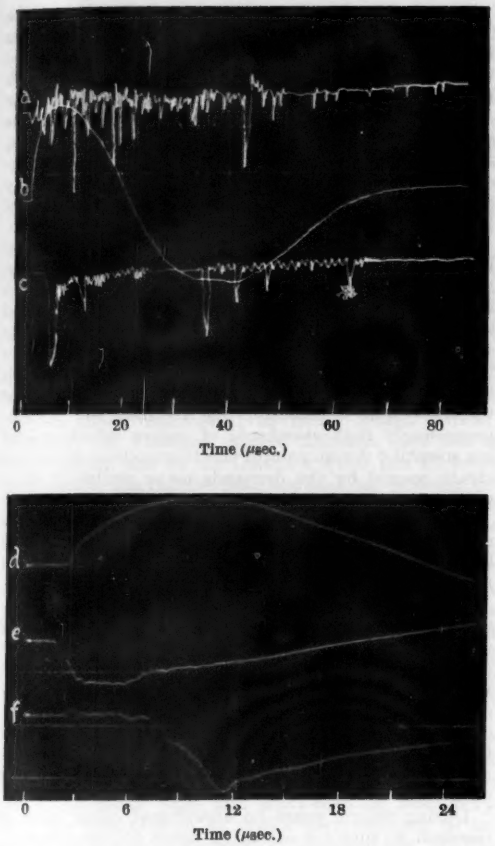


Fig. 3. Multiple-beam oscilloscope traces as a function of time of: *a*, neutron capture γ-rays from a cadmium-loaded liquid scintillator; capture half-life ~ 30 μsec.; *b* and *d*, gas current; *c*, proton recoils from a fast scintillator; *e*, secondary voltage; *f*, intensity of silicon line, 4128 A.

witness the great sensitivity of instability neutrons to impurities[8–10].

We think it fairly safe to say that at any rate the fringe of thermonuclear temperatures of approximately 500 eV. is being approached. The principal anxiety arises from the fact that it is precisely at this point that conditions are ideal for simulation of a thermonuclear reaction by a Fermi mechanism[11]. Reflexion of deuterons from magnetic disturbances travelling at the Alfvén speed $\sqrt{B^2/4\pi\rho}$, give deuterons in the 12-keV. energy-range after very few reflexions, yet a small fraction of the gas, 0·6 per cent with this energy, equals the thermonuclear yield of a plasma at 600 eV. Nor is it easy to distinguish the two experimentally. Refined nuclear emulsion techniques could do it, but would require several thousand discharges to accumulate enough intensity at the present output-level of Perhapsatron *S*-3. Comparison of yields with DD and DT gas fillings can also be used, but requires more reproducibility than prevails at present.

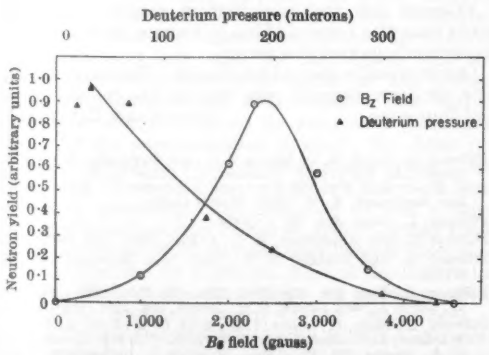


Fig. 4. Neutron yield versus initial longitudinal B_z magnetic field and deuterium gas pressure. The experimental points are averages of several runs with variations of a factor 3 in neutron yield

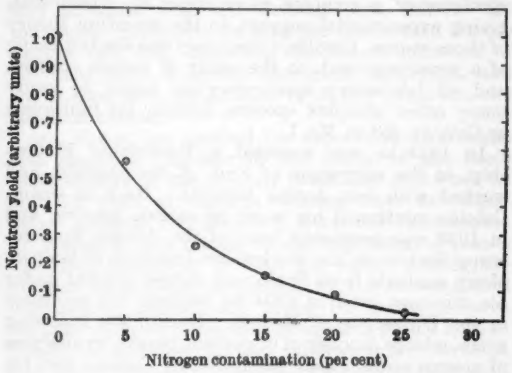


Fig. 5. Neutron yield versus percentage nitrogen contaminant (mean of several runs)

We acknowledge with pleasure the co-operation of L. Burkhardt and R. Lovberg for scientific contributions to the experiment, of R. Dike for engineering design, of A. Schofield for electrical design, of A. Roensch for inspired precision glass-blowing with difficult materials, and of J. M. B. Kellogg for co-operation in the division.

We are indebted to the Atomic Energy Research Establishment, Harwell, for assistance in handling this paper for publication simultaneously with the ZETA experiments (which, incidentally, preceded this work by several months).

This work was performed under the auspices of U.S. Atomic Energy Commission.

[1] Kruskal, M., and Schwarzschild, M., *Proc. Roy. Soc.*, A, **223**, 348 (1954).
[2] Kruskal, M., and Tuck, J. L., Los Alamos Report 1716, *Proc. Roy. Soc.* (to be published).
[3] Tayler, R. J., *Proc. Phys. Soc.*, B, **70**, 31 (1957).
[4] Rosenbluth, M., Los Alamos Report 2030, Proc. Venice Conference (1957).
[5] Shafranov, V. D., *Atomnaya Energiya*, **5**, 709 (1956).
[6] Burkhardt, L. C., Lovberg, R. H., and Phillips, J. A., *Nature* (p. 224 of this issue).
[7] Burkhardt, L. C., Houseker, J., Karr, H., and Lovberg, R. (private communication).
[8] Artsimovich, L. A., Andrianov, A. M., Dobrokhotov, E. I., Lukyanov, S. Yu, Podgornyi, I. M., Sinitsyn, V. I., and Filippov, N. V., *Atomnaya Energiya*, **3**, 84 (1956).
[9] Anderson, O. A., Baker, W. R., Colgate, S., Gilbert, F. C., Ise, J., Pyle, R. V., and White, R. S., Proc. Venice Conference (1957).
[10] Dunaway, R. E., and Phillips, J. A., Los Alamos *LADC* 2943 (to be published).
[11] Fermi, E., *Phys. Rev.*, **75**, 1169 (1949).

OBITUARIES

Prof. M. A. Catalán

Miguel Antonio Catalán was born at Saragossa in Spain on October 9, 1894, and died at Madrid on November 11, after a brief and unexpected illness, at the age of sixty-three.

Following graduation at the University of Saragossa, Catalán received his Ph.D. degree with honours at Madrid in 1917 and started research work on spectroscopy in the laboratory for advanced studies of the Department of Physics. With a Spanish fellowship, he studied in England under Prof. Alfred Fowler's direction in 1920–21. During this stay at the Imperial College of Science and Technology, Catalán made his most important contribution to spectroscopy with the discovery of multiplets in the arc spectrum of manganese. For the first time, the spectrum of a complex atom could be interpreted, giving experimental support to the quantum theory of those atoms. Catalán's discovery was the beginning of a novel approach to the study of atomic spectra, and all laboratory spectroscopists began to study many other complex spectra, looking for multiplets as Catalán did in Mn I.

In 1923 he was awarded a Rockefeller Fellowship, at the suggestion of Prof. A. Sommerfeld, and worked with him during 1923–24. Back in Spain, Catalán continued his work on atomic spectra, and in 1930 was promoted head of the Atomic Spectroscopy Section at the Rockefeller Institute of Madrid. Many students from Spain and abroad worked under his direction, and in 1934 he became full professor in the University of Madrid. During the following years, a large number of important papers on analyses of atomic spectra were published by Catalán and his students; thus, very soon his school was well known and appreciated all over the world. Invited by the American Philosophical Society, he worked in the United States during 1947–48 and again in 1950 and 1951. In 1950 he was appointed chief of the Department of Spectroscopy in the newly created Institute of Optics, Madrid; and in 1952 he was elected to the Joint Commission for Spectroscopy of the International Council of Scientific Unions. His important contributions to the field of atomic spectroscopy were also rewarded in Spain when he was elected member of the Real Academia de Ciencias in 1955. At the invitation of South American universities, Catalán lectured in Argentina and Venezuela in 1956 and 1957. Up to the date of his illness, he was still very active and productive; thus his death surprised and shocked all his friends.

Prof. Catalán was not only an eminent spectroscopist; he was also a great teacher and he enjoyed lecturing to students or to any type of audience. Teaching was really his hobby. He had the rare ability of making simple and understandable the most difficult subjects, and he liked these outside activities so much that, apart from his lectures at the University, he always instructed groups of high-school students.

Catalán was friendly and helpful to everyone. He was liked and admired by all who knew him, and he will be remembered with affection by his many friends and colleagues.

R. Velasco

Prof. Louis Pillemer

Louis Pillemer, well known to many immunologists in Great Britain, died unexpectedly on August 31, 1957, at the age of forty-nine years.

Born in South Africa, Pillemer spent all but the first year of his life in the United States. He took a B.S. degree at Duke University in 1932, went to Western Reserve University in 1935 and took his Ph.D. there in 1938. In 1944 Pillemer worked at the Army Medical School in Washington. When he returned to Western Reserve University he became, in quick succession, associate professor of immunochemistry (1946) and professor of biochemistry (1950) without teaching duties at the Institute of Pathology.

His association with W. A. Perlzweig at Duke University, with E. E. Ecker, who was his chief at Western Reserve University for many years, and with E. J. Cohn at Harvard, undoubtedly influenced his experimental approach and orientation, but the direction and drive of his endeavours flowed from his own stormy and original personality and from the intuitive faculties of his mind. When one came to know him intimately, one realized the uncompromisingly high standards of values which guided his scientific decisions and the tremendous emotional strain caused by the demands he so pitilessly made on himself.

Pillemer's experimental work ranged from studies of protein specificity[1] and complement[2] to clinical biochemistry[3] and to the purification of toxins and toxoids[4].

Pillemer made contributions of fundamental significance in several directions, but his choice of research projects was usually strongly weighted by their potential practical value. Purification studies were aimed at an improved combined vaccine of diphtheria–tetanus–pertussis; the later development of a pertussis vaccine by ultrasonic disintegration of the microbes followed by absorption of the protective antigen on human red cell stroma continued this endeavour[5].

During recent years he abandoned other lines of research to turn his attention again to complement; soon 'Properdin' was discovered by him and his small band of enthusiastic and able colleagues and assistants. 'Properdin', acting with components of complement and magnesium ions, was shown to be part of the constitutive defence mechanisms of the mammalian organism and to mediate the destruction of certain micro-organisms and abnormal red cells[6]. By the time of Pillemer's death, it was clear that the discovery of 'Properdin' represented an important milestone in the study of the non-induced defence mechanisms against infection.

Pillemer felt that the highest award a scientist could gain was respect of his colleagues for his work—he certainly gained this award.

Our sympathy goes to his family. He leaves a wife, who as Jean Burrell[7] was among his collaborators, and four sons.

Bernhard Cinader

[1] Pillemer, L., Ecker, E. E., Myers, V. C., and Muntwyler, E., *J. Biol. Chem.*, **123**, 365 (1938). Pillemer, L., Ecker, E. E., and Wells, J. R., *J. Exp. Med.*, **69**, 191 (1939). Pillemer, L., Ecker, E. E., and Martiensen, E. W., *ibid.*, **70**, 387 (1939).

[2] Pillemer, L., *Chem. Rev.*, **33**, 1 (1943).

[3] Pillemer, L., and Hutchinson, M. C., *J. Biol. Chem.*, **158**, 299 (1945).

[4] Pillemer, L. and Robbins, K. C., "Ann. Rev. Microbiol.", **3**, 265 (1949).

[5] Pillemer, L., *Proc. Soc. Exp. Biol. Med.*, **75**, 704 (1950). Pillemer, L., Blum, L., and Lepow, I. H., *Lancet*, **266**, 1257 (1954).

[6] Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C., *Science*, **120**, 279 (1954). Pillemer, L., and Ross, O. A., *Science*, **121**, 732 (1955). Pillemer, L. Schoenberg, M. D., Blum, L., and Wurz L. *Science*, **122**, 545 (1955).

[7] Pillemer, L., Burrell, J. I., and Ross, O. A., *Science*, **106**, 36 (1947). Burrell, J. I., Robbins, K. C., and Pillemer, L., *Science*, **108**, 311 (1948).

NEWS and VIEWS

Hydraulics Research : Sir Claude Inglis, C.I.E., F.R.S.

Sir Claude Inglis is retiring from the post of director of the Hydraulics Research Organization of the Department of Scientific and Industrial Research. According to "Who's Who", Sir Claude was born in 1883 ; those who are familiar with him and with his work will read this statement with some incredulity, for it implies that at the date of his appointment as the first director of the Organization he was already some sixty-three years of age. Although so many fundamental contributions to the subject of hydraulic scale models had been made by British engineers (notably Osborne Reynolds and A. H. Gibson), facilities for such investigations, especially in the field of loose-boundary hydraulics, then lagged far behind those established in many places abroad. Happily, the situation is now different, and this is very largely due to Sir Claude's quiet but effective persistence, his infinite patience and his capacity for continuous hard work ; all combined with his specialized knowledge and the experience of his earlier directorship of the Indian Waterways Experiment Station at Poona. The Department of Scientific and Industrial Research now has a magnificent Hydraulics Research Station at Wallingford (a site chosen by Inglis), which includes a main experimental hall with a floor area of 60,000 sq. ft. Before joining the Department, Sir Claude had comparatively little experience of tidal models ; nevertheless, he has since directed several notable tidal model investigations of considerable complexity. A remarkable feature of his directorship has been his enthusiasm for the development of new measuring devices, the design of novel tide- and wave-making machinery and the exploitation of radioactive isotopes as an aid in tracing the movement of sand and silt. At the same time, he has maintained his interest in the basic mechanics of the regime of channels and the meandering of rivers.

Mr. Fergus H. Allen

Mr. Fergus H. Allen, who has been appointed to succeed Sir Claude Inglis as director of the Hydraulics Research Station at Wallingford, has earned widespread respect and confidence during tenure of the office of assistant director. The site of the Station was only acquired in 1949, and the design and construction of buildings and equipment there have clearly demanded strenuous efforts by all concerned and not least by Mr. Allen, who has shared the responsibility for many of the investigations undertaken both at the Station itself and on other premises, and has at the same time shouldered many of the administrative duties. Mr. Allen has visited hydraulics research laboratories abroad and has been the author or co-author of several publications, including "The Regimen of the Thames Estuary as affected by Currents, Salinities and River Flow", a paper which aroused considerable discussion when it was read by Inglis and Allen at the Institution of Civil Engineers in May 1957. At meetings of the Hydraulics Research Board of the Department of Scientific and Industrial Research and at conferences held by bodies such as the International Association for Hydraulic Research and the Permanent International Association of Navigation Congresses, Mr. Allen has made a very favourable impression by his grasp of the subjects under discussion and by his capacity for lucid exposition. The Hydraulics Research Station may look forward with confidence to continuing progress under his direction.

Irrigation Research Station, Griffith : Mr. E. R. Hoare

Mr. E. R. Hoare has been appointed as officer-in-charge of the Commonwealth Scientific and Industrial Research Organization, Irrigation Research Station, Griffith, New South Wales. Mr. Hoare comes from the United Kingdom Institute of Agricultural Engineering, Silsoe, Bedfordshire. He has been in charge of the Horticultural Engineering Department, which has made important contributions to research on plant cultivation. He graduated in mechanical and electrical engineering at the University of London in 1934. For eleven years he was research engineer and physicist with the General Post Office. He undertook important services for the Armed Forces during the War, including the synchronization of all radar stations on the east coast of Britain, and the design and installation of D-day cross-channel communications.

Mr. Hoare has applied his experience as a radio and electrical engineer to the study of the basic requirements of the growing plant. In this work he and his team of research workers have developed many original and sensitive electronic instruments for assessing such factors as the respiration of plants and their sensitivity to light and heat. Among the projects successfully developed by the Horticultural Engineering Department under his guidance have been the study of the uptake of water in plants both in glasshouse and open ground cultivation ; cultivation and tractor problems ; plant-culture under glass with special reference to heat requirements ; crop- and pest-spraying problems ; study of light, radiant energy and radioactivity in relation to plant growth.

Giovanni Alfonso Borelli (1608–79)

Mathematician, astronomer, physicist and physiologist, Giovanni Alfonso Borelli was the son of a soldier and was born at Naples 350 years ago on January 28, 1608. A pupil of Galileo, he became professor of mathematics at Messina in 1649 and seven years later, at the invitation of Ferdinand II, Duke of Tuscany, went to Pisa. There he met Malpighi, whom he taught mathematics and physics and from whom he acquired an interest in physiology. Borelli looked upon locomotion, respiration and digestion as mechanical processes, describing digestion as the result of a mechanical trituration of the food within the stomach. He taught, erroneously, that contracting muscle increases in bulk due to fermentation in muscle, and believed that the nerve supplying the muscle discharged a fluid, *succus nerveus*—his substitute for Galen's 'animal spirits'. His neurogenic theory of the heart beat, revived by J. J. C. Legallois in 1812, held the field against the myogenic theory until modern times. Borelli's "De motu animalium", an enormous work in two volumes, was published posthumously in 1680–81. His pseudonymous letter, "Del movimento della cometa apparsa il mese di decembre 1664" (1665), contains the first suggestion of a parabolic path, and his "Theorica mediceorum planetarum ex causis physicis deducta" (1666) deals with the influence of attraction on the satellites of Jupiter. In 1679 he invented a diving apparatus capable of altering the specific

gravity of the diver. Borelli returned to Messina in 1668 and in 1674 retired to Rome, where he lived under the protection of Queen Christina of Sweden. A morose, obstinate and quarrelsome man, he died of pleurisy on December 31, 1679.

The Commonwealth Trans-Antarctic Expedition

The trans-Antarctic survey party led by Dr. Vivian Fuchs reached the South Pole on January 20, where they were met by Sir Edmund Hillary and Rear-Admiral G. Dufek, commander of the American naval base at the Pole. The expedition, which consists of Dr. Fuchs and his team of eleven men, four 'Snocats' and a 'Weasel', with two dog teams and sledges, left Shackleton Base for South Ice on the Weddell Sea on November 24 and started from South Ice for the Pole on December 21. Throughout the journey the expedition has conducted a programme of scientific observations including seismic soundings every thirty miles, gravimetric readings every fifteen miles and meteorological observations every three hours, in connexion with the International Geophysical Year. It is reported that Dr. Fuchs proposes to continue his journey across the Antarctic continent to Scott Base on the Ross Sea using the depots of food and fuel established by Sir Edmund Hillary's earlier supporting party.

Tenth Anniversary of British Ocean Weather Ships

In August 1957 British ocean weather ships completed ten years of service in the North Atlantic. The first, *Weather Observer*, arrived at station 'Juliett' (53° 50′ N., 18° 40′ W.) on August 5, 1947, and transmitted her first observation to the Central Forecast Office, Dunstable, on that day, thereby inaugurating the active participation of the United Kingdom in the North Atlantic Station Agreement. The record of the ten years work is summarized by Comdr. C. E. N. Frankcom, marine superintendent of the Meteorological Office, in an article in the issue of the *Meteorological Magazine* of October 1957. Besides the usual surface observations, regular upper air ascents with radio-sonde and radar-wind balloons have been made, a difficult task in the high winds so often encountered at the ocean weather stations.

In addition to performing their meteorological work, the four ships have provided navigational assistance to aircraft, carried out oceanographic research, and been used for researches by other institutions, for example, in seismological work by the University of Cambridge. Ten of the marine staff have served throughout the decade and one meteorologist for six years. The ships employed, converted "Flower"-class naval corvettes, are showing wear after their war and ocean-station work and one of them is to be replaced by a larger "Castle"-class frigate now being converted for its new duties. The new vessel, which may be followed by others of the same type, will be much more roomy and comfortable.

U.S. National Astronomical Observatory

The National Science Foundation has entered into a contract with the Association of Universities for Research in Astronomy, Inc., for the construction, operation and maintenance of a U.S. national astronomical observatory. The Association, which has its principal office in Phoenix, Arizona, comprises the following Universities: California, Chicago, Harvard, Indiana, Michigan, Ohio State and Wisconsin. These Universities joined together to constitute a management group for the new observatory, because they have had experience in operating large observatories and have big programmes of research and graduate instruction in astronomy. As the project develops, it is expected that other universities and individuals will join the Association. The Board of Directors of the newly formed corporation consists of a scientific and an administrative representative of each of the seven participating universities, as follows: *California:* Dr. C. D. Shane, director, Lick Observatory; Mr. J. M. Miller, assistant vice-president, business affairs; *Chicago:* Dr. G. P. Kuiper, director, Yerkes Observatory; Mr. W. B. Harrell, vice-president, business affairs; *Harvard:* Dr. D. H. Menzel, director, Harvard College Observatory; Mr. Edward Reynolds, administrative vice-president; *Indiana:* Dr. F. K. Edmondson, chairman, Department of Astronomy; Mr. J. A. Franklin, vice-president and treasurer; *Michigan:* Dr. R. R. McMath, director, McMath–Hulbert Observatory; Mr. G. L. Lee, jun., controller; *Ohio State:* Dr. P. C. Keenan, professor of astronomy; Mr. C. F. Miller, controller; *Wisconsin:* Dr. A. E. Whitford, director, Washburn Observatory; Mr. A. W. Peterson, vice-president. The corporation appointed Dr. McMath president, Dr. Edmonson as vice-president, and Mr. J. M. Miller as secretary.

The new observatory, when completed, will be open for research to all qualified astronomers. Under the terms of the contract, it is hoped to equip the Observatory initially with a 36-in. and an 80-in. telescope. The National Science Foundation's appropriation for the fiscal year 1958 includes an item of 3·1 million dollars for construction of an optical observatory on a site which is to be selected as a result of extensive tests. The Foundation earlier awarded grants totalling approximately 800,000 dollars to the University of Michigan to support studies leading to the establishment of such an observatory (see *Nature*, **178**, 837; 1956). After extensive studies under the direction of Dr. Aden B. Meinel, executive secretary of the Foundation's advisory panel for the observatory, the choice of site was narrowed to five locations: Kitt Peak (6,875 ft.), 40 miles south-west of Tucson, Arizona; Summit Mountain (7,500 ft.), 11 miles south of Williams; Chevalon Butte (6,950 ft.), 40 miles south of Winslow; the Hualapai Mountains (7,350 ft.), 13 miles south of Kingman; and Junipero Serra (5,800 ft.), 21 miles west of King City, California. A special committee, under the chairmanship of Dr. C. D. Shane, director of Lick Observatory, recently spent some time in Arizona reviewing the situation. As a result, site studies will be continued at Kitt Peak and Hualapai and at a new location on Mormon Mountain (8,440 ft.), 35 miles south of Flagstaff, but discontinued elsewhere because recent work has shown that the other sites are unsuitable. Temperature and wind velocity will continue to be measured and seeing conditions tested at the three remaining sites. Studies are expected to be completed by about June 30, 1958.

British Group for Computation and Automatic Control

The British Conference on Automation and Computation is being organized in three sections, as follows: (*A*) the British Group for the Engineering Applications of Automation; (*B*) the British Group for Computation and Automatic Control; and (*C*) the

British Group for the Sociological and Economic Aspects of Automation Techniques (*Nature*, **179**, 948; 1957). At a meeting held on December 20, Group *B*, the British Group for Computation and Automatic Control, was formally constituted, comprising as its members twenty-three professional and learned institutes and societies with interests in these fields.

The objects of the Group are to foster the development and applications of automatic controls, computing and data-processing equipment and programming techniques; to afford a common meeting ground for the adhering organizations; to maintain liaison with other Groups of the British Conference on Automation and Computation and with corresponding national committees of other countries; and to encourage and co-ordinate the presentation at international conferences of British papers on subjects falling within the purview of the Group. Mr. T. E. Goldup (president of the Institution of Electrical Engineers) was elected chairman of the Group, with Messrs. J. F. Coales (Society of Instrument Technology) and E. M. Renals (Institute of Cost and Works Accountants) as vice-chairmen. Mr. J. D. Green (Institute of Chartered Accountants in England and Wales) was elected honorary treasurer, and Mr. W. Bamford (Institution of Electrical Engineers) honorary secretary, and the offer of the Institution of Electrical Engineers to provide secretarial services for the Group was accepted. The chairman, the honorary secretary and one vice-chairman (Mr. E. M. Renals) were chosen to act as the representatives of the Group on the general committee of the British Conference on Automation and Computation, which will also comprise representatives of the two remaining Groups now in process of formation. The constitution of the Group provides for the election of additional member societies: those interested should communicate with the honorary secretary of the Group, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2.

Transparent Magnetic Oxides

THE work going on in Prof. L. Néel's laboratory in Grenoble on ferrimagnetism in rare earth iron garnets has recently been extended in the Bell Telephone Laboratories by an investigation of their optical resonance behaviour carried out by J. F. Dillon, jun., using single crystals grown by J. W. Nielsen. These materials are remarkable in that some of them are transparent. Consequently, their Faraday rotation can be conveniently investigated, and it is found that it amounts to several degrees per mil of thickness, so that domains within the crystals can be made clearly visible, enabling the domain structure to be studied over a wide range of temperature and field conditions. Yttrium iron garnet has been most completely studied so far. It has a Curie temperature of 545° K. and a spontaneous magnetization, at 0° K. and infinite field, of 4·96 Bohr magnetons per molecule, which is close to the theoretical value of 5·0. This magnetization results from super-exchange interactions through the O^{2-} ions between Fe^{3+} ions in crystallographically different positions in the cubic lattice. The fact that yttrium iron garnet contains magnetic ions with but a single valence is of great interest, and X-ray and neutron-diffraction studies have shown that, unlike the ferrites, interactions between identical magnetic ions wholly occupying two different crystallographic sites are responsible for ferrimagnetism in the garnet structure.

Systematics and Biology of Ungulates

ABOUT sixty zoologists from Belgium, Denmark, France, Germany, Great Britain, Holland, Italy, Russia, Switzerland and the United States, attended a three-day colloquium in Paris on the "Systematics and Biology of the Ungulates" during October 23–26. The colloquium was arranged by the Comité de Direction et d'Administration of the journal *Mammalia* and the (Benelux) Société pour l'étude et la protection des mammifères, under the presidency of Prof. E. Bourdelle, with Prof. Roger Heim as président d'honneur, and Dr. Jean Dorst as secretary-general. After a reception at the Parc zoologique de Vincennes on the evening of October 23, the following three days were fully occupied in the presentation and discussion of some thirty papers embracing many aspects of the subject from classification and conservation to blood groups, placental structure, animal behaviour, and the biology of particular species. The text of these papers, together with some others the authors of which were unable to be present at the colloquium, will be published in full in *Mammalia*. The members of the colloquium agreed, as was plain from the speeches at the concluding dinner where they were the guests of the management of *Mammalia*, that the meeting was most valuable and stimulating not only in its formal business but equally in the opportunity it gave to zoologists working on the Mammalia for informal discussion and exchange of ideas. It is greatly to be hoped that organizations in other countries will follow the example set by their colleagues of France, and will arrange similar international meetings for the benefit of zoologists interested in the study of the Mammalia.

Cytological Techniques and the Apical Meristem

THE reactivity of the shoot apical meristem as ascertained by cytological and other techniques, in particular by French observers led by Plantefol and Buvat, has been discussed by Wardlaw (*New Phytol.*, **56**, 221; 1957). The French school places great emphasis on the initial or meristematic ring as the active region of the apical meristem, particularly in the inception of leaf primordia and phyllotaxis. The conclusion reached in the paper under consideration is that the cytological investigations now being so vigorously pursued by the French workers and their adherents are to be welcomed as contributing new facts about the distribution of nuclear and cytoplasmic proteins in different regions of the apex; and it should be recognized that the theories and ideas which have been expounded, whether or not they are validated by further work, have given a new impetus and direction to the study of the shoot apex. The results that have so far accrued from this approach, however, tend to forfeit some of their value and objectivity, partly because of the *a priori* complete acceptance and, indeed, over-emphasis, on the part of the protagonists and their followers, of the validity of the underlying theory of phyllotaxis and apical organization, with a consequential tendency for the observations to be somewhat selective in character, and partly because the new observations have not been related to the well-substantiated body of evidence on apical ontogeny, organization and reactivity that has accrued from experimental and other investigations. Lastly, in considering the views of the French workers and criticisms of these views, it is well to bear in mind how very little is yet known about the physiology and biochemistry of the apex.

New facts, however, can be sought and they may decide the controversial issues.

Peaceful Uses of Atomic Energy

A major symposium on the peaceful uses of atomic energy will be held in Sydney during June 2–6. The symposium is being sponsored by representatives of the universities, professional institutes, industrial firms, power authorities, Commonwealth and State departments and the Australian Atomic Energy Commission. The symposium will be a forum for the discussion of technical and scientific aspects of the peaceful uses of atomic energy. Papers will be presented by overseas scientists as well as many Australian contributors. Particular attention will be given to the part atomic energy can play in the development of Australia. There will be several public lectures and a special atomic energy exhibition, to show developments in uranium mining, research into nuclear power and the industrial uses of radioactive isotopes. Further information can be obtained from the Symposium General Secretary, A.A.E.C. Research Establishment, Private Mail Bag, Sutherland, New South Wales, Australia.

University News: London

Dr. F. G. Foster, lecturer in statistics in the London School of Economics and Political Science, has been appointed to the University readership in statistical computing tenable at that School.

Manchester

The board of Unilever has given to the Manchester College of Science and Technology £21,000 under a seven-year deed of covenant. Out of this, £2,000 is to be allocated to the staff college, the balance to be divided equally between the Chemistry and Chemical Engineering Departments.

Announcements

A discussion on "Experimental Results with the ZETA Apparatus on Controlled Thermonuclear Reactions" will be opened by Sir John Cockcroft in the rooms of the Royal Society on February 5 at 3 p.m.

Mr. John Bunyan, Leverhulme Research Fellow, St. Mary's Hospital Medical School, Paddington, has been elected president of the Royal Microscopical Society; he will hold office for two years.

The Medal of the New York Academy of Medicine has been awarded to Dr. Alphonse R. Dochez for his distinguished service in medicine. After graduation from the Johns Hopkins Medical School, Dr. Dochez was appointed to the staff of the Rockefeller Institute, where, with Gillespie, he established the biological classification of pneumococci into specific types. During the First World War, Dr. Dochez worked on the serological differentiation of hæmolytic streptococci causing respiratory diseases, and later devoted some years to virus studies of the common cold.

Mr. Lewis T. Wright, general secretary of the Amalgamated Weavers' Association, has been appointed to the Council for Scientific and Industrial Research in succession to Mr. W. L. Heywood, who has been appointed a member of the Restrictive Practices Court. Mr. Wright is chairman of both the Production and Scientific Advisory Committees of the Trades Union Congress and is also chairman of the British Productivity Council.

Dr. M. E. Foss has been appointed head of the Department of Chemistry and Metallurgy, Coventry Technical College, in succession to Dr. H. C. Smith, who has been relieved of that office in order to take up the full-time post of vice-principal of the College, of which he has been deputy-principal for the past five years. Dr. Foss, who is vice-chairman of the Bristol Section of the Royal Institute of Chemistry, was senior lecturer in organic chemistry at Gloucester Technical College where he had been on the staff for six years. Prior to that he was lecturer in biochemistry at Guy's Hospital Medical School.

The sixty-third annual congress of the South-Eastern Union of Scientific Societies will be held in Reading, meeting in the University of Reading, during April 9–11. The president-elect is Prof. H. L. Hawkins. Further information can be obtained from the hon. local secretary, Mrs. A. Hasker, Clarence Lodge, 93 London Road, Reading.

The Metal Physics Committee of the Institute of Metals is organizing a one-day symposium on "Metallurgical Aspects of Semi-Conductors", to be held on February 25 at the College of Technology, Gosta Green, Birmingham. Further information can be obtained from the Secretary, Institute of Metals, 17 Belgrave Square, London, S.W.1.

The annual general meeting of Aslib (Scottish Branch) will be held on February 7 in the Scottish Central Library, Lawnmarket, Edinburgh. The chairman will be Dr. W. M. Cumming, and there will be lectures on "United States Information Services to Industry in the United Kingdom" by Miss Margaret Haferd, and "Scientific Information in the Modern World" by Dr. J. C. Malloch. In the afternoon there will be a symposium on "Librarians Here and There".

The second Australian Conference in Radiation Biology will be held at the Cancer Institute, Melbourne, Australia, during December 15–19. Papers on relevant subjects are invited, and titles and a 250-word abstract should be in the hands of the convener by July 31. Further information can be obtained from Dr. J. H. Martin, Physics Department, Cancer Institute Board, 483 Lt. Lonsdale Street, Melbourne, Victoria, Australia.

The Ministry of Agriculture, Fisheries and Food is offering the following scholarships for rural workers, or to the sons and daughters of rural workers, of seventeen years of age or more: twenty senior scholarships tenable at universities or colleges, for degree or diploma courses in an agricultural subject or in veterinary science; and thirty junior scholarships, with six extensions for existing holders, tenable at farm institutes for one-year courses in agriculture, horticulture, dairying or poultry husbandry.

Further details and application forms (to be completed by February 28) may be obtained from the Secretary, Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1, or from education offices of County Councils.

The International Council of Scientific Unions Secretariat has removed to a new address at Palais Noordeine, The Hague, Netherlands.

Erratum. In the communication entitled "Antimitotic Activity of 5-Tertiary Butyl Benzimidazolone" in *Nature* of December 21, p. 1428, from paragraph 3 onwards, for "5-tertiary benzyl benzimidazolone" read "5-tertiary butyl benzimidazolone".

NEWER PREPARATIVE METHODS IN ORGANIC CHEMISTRY

A SYMPOSIUM was held jointly by the Chemical Society and the Fine Chemicals Group of the Society of Chemical Industry on "Newer Preparative Methods in Organic Chemistry" at the Chemistry Department, University College, London, on November 7. The meeting took the form of two sessions in the afternoon at each of which three papers were read; the reading of papers was followed by a dinner in the evening, also at University College. The afternoon meetings were attended by nearly 400 chemists and the evening dinner by 116. Papers were read on "Dehydrogenation with Manganese Dioxide" by Dr. R. M. Evans, "Amine Oxidations" by Prof. H. B. Henbest, "Organic Oxidations with Hexavalent Chromium" by Dr. R. Slack, "Mixed Anhydrides as Synthetical Reagents" by Prof. G. W. Kenner, "Some Reactions between Metal Carbonyls and Acetylenic Compounds" by Dr. M. C. Whiting and "Some Applications of Metal Carbonyls in Organic Chemistry" by Dr. P. O. Lenel.

The meeting was opened by Prof. E. L. Hirst, who mentioned that this was the first joint meeting between the Chemical Society and the Fine Chemicals Group and expressed his hope that it would be a successful one. Dr. B. A. Hems took the chair at the first session and Prof. E. R. H. Jones presided over the second.

The purpose of the meeting was to discuss the applications of some reagents that have recently come into prominence in preparative chemistry and of which the scope and details of experimental methods may not yet be widely known. The authors of the papers had themselves had extensive personal experience of these reagents and could point to the theoretical and practical significance of various methods of using them.

Dehydrogenation by manganese dioxide was discussed by Dr. Evans. When this reagent is used for dehydrogenation the method of preparation and the physical form of the oxide appear to have a considerable effect on its reactivity. One form, prepared under strongly alkaline conditions, appears to be the best for dehydrogenating unsaturated primary or secondary alcohols to $\alpha:\beta$-unsaturated aldehydes or ketones, whereas an acidic form appears to be better for dehydrogenating unsaturated ketones, at least in the sterol group. Dr. Evans pointed out that manganese dioxide brought about many other oxidizing reactions under more vigorous conditions, but that selectivity was then lost. The most useful applications of this reagent lay with specially active forms of the oxide used in mild conditions on polyene or polyenyne alcohols that were difficult to oxidize to the corresponding aldehydes or ketones by other methods.

The dehydrogenation of amines to give unsaturated compounds was discovered many years ago but has received very little application in general preparative chemistry. It is to be expected that the work that Prof. Henbest and his colleagues are doing on the subject will bring this potentially useful reaction to the notice of a wider audience.

He mentioned in his paper that tertiary amines are generally regarded as being chemically rather unreactive, but pointed out that this is not so. Dehydrogenation with mercuric acetate under mild conditions in dilute acetic acid gives enamines. Sometimes in a heterocyclic ring system complete dehydrogenation to the aromatic ring will take place, but often partial dehydrogenation can also occur. He compared this with the action of manganese dioxide on tertiary amines, where again enamines are formed under mild conditions, but this is often accompanied or followed by oxidative cleavage of the double bond to give amides. Diethylaniline gives a mixture of ethylvinyl aniline and ethylformanilide.

The third of the oxidizing agents discussed, chromic oxide, is, of course, widely used for a variety of purposes. It may be applied to both dehydrogenations and oxidations by a diversity of methods the theoretical and practical significance of which is not yet thoroughly understood. Differences undoubtedly exist about the effectiveness with which these different techniques can be used on different substrates, and although it is probable that a common theoretical background exists, it is nevertheless true that much has yet to be learnt about the best method to be used on any particular type of substance. Dr. Slack pointed out that oxidations with chromic oxide in acetic acid were often not stoichiometric, that chromic oxide could still remain, even in the presence of unoxidized substrate, because its oxidizing action was inhibited by the presence of lower valency forms of chromium. He added that one function of the sulphuric acid, sometimes added to these reactions, is to overcome this inhibition. He compared the uses of chromic oxide as its pyridine complex or tertiary butyl ester and finally pointed to the existence of some other hexa- and penta-valent chromium complexes such as acetyl chromic acids or complex salts derived from trichloro oxo-chromium (V) that had not yet been investigated by organic chemists but might repay some attention.

These three papers composed the first session of the meeting and were followed by three on condensation reagents.

Mixed anhydrides have come very much into prominence during the past few years for condensations involving carboxyl groups where, because of the delicacy of either the starting materials or the final product, mild reaction conditions are required.

Another advantage that mixed anhydrides have is that one of the partners is usually derived from a small and relatively cheap molecule, and their use avoids the wasteful regeneration of a molecule of the larger and perhaps much less accessible acid. Prof. Kenner pointed out that in a mixed anhydride a cleavage reaction could theoretically take either of two directions and he discussed the relative activities of various groups showing that the attack of a nucleophilic reagent was determined by the relative electrophilic character of the two parts of the anhydride. This, however, was not the only factor affecting reaction; the pK of the acids taking part, steric hindrance and the co-ordination number at the electrophilic centres also played a part in determining which of the two reaction products would predominate. Mixed anhydrides derived from chloroformic ester, phosphoric and sulphuric acids or their esters had shown themselves to be extremely useful in the synthesis of peptides and nucleosides.

The last two papers were on metal carbonyls. Frequently to-day, new preparative methods involve the use of reagents that a few years ago were considered too difficult or too dangerous to handle because of the risks of explosion, fire or toxicity. The hazards of using acetylene under pressure and the toxicity of carbonyls frequently cause these reagents to be avoided and it is hoped that these papers, in which safe methods of handling were discussed by the authors, may alleviate some of these fears.

Dr. Whiting stressed the usefulness of nickel carbonyl in reacting with acetylenes having a terminal triple bond to give α-substituted acrylic acids, a type of compound otherwise rather inaccessible. These reactions are carried out in acetic acid at moderate temperature, with excess carbonyl which is not difficult to handle provided that its transfer from vessel to vessel is carried out under conditions involving only a very slight degree of exposure to air, and provided that excess is removed from the reaction mixture by co-distillation with ether. The distillate is immediately disposed of and leaves a reaction mixture that can safely be handled in the usual way. Even so, both he and Dr. Lenel pointed out that although these substances could be handled safely they still had to be treated with considerable respect. Dr. Whiting also went at some length into the mechanisms of these reactions. He was followed by Dr. Lenel, who dealt mostly with the industrial applications of carbonyls in reaction with acetylenes to give unsaturated acids, or with olefines to give saturated acids. Cobalt carbonyl catalyses the important reaction of carbon monoxide and hydrogen with olefines to give aldehydes which can then be reduced to alcohols by hydrogenation. The industrial preparation of alcohols and acids by these reactions is now carried out on a very large scale involving many thousands of tons of product per annum, though the reactions necessitate fairly high temperatures and pressures. Several other carbonyls of transitional elements are known, though they have not yet achieved prominence in an industrial sense; they are of profound interest to the inorganic and theoretical chemist because their constitutions throw new light on electronic structures.

This was, as Prof. Hirst pointed out, the first joint meeting of the Chemical Society and the Fine Chemicals Group of the Society of Chemical Industry, and it is very much to be hoped that it will be followed by others. The members of the Fine Chemicals Group are predominantly interested in the preparation of complex chemicals in a high degree of purity: perfumes, photographic sensitizers, drugs, vitamins and hormones, for example. These processes often involve twelve to twenty stages that, if they are to be carried out efficiently, demand the use of the most up-to-date knowledge and techniques available. This Group, of all the special groups in the Society of Chemical Industry, is the one that has the strongest kinship with the Chemical Society. With the rapidly increasing activity in industrial chemistry of all kinds and the rapid extension of industrial technical staffs, joint meetings of this kind can be a valuable aid to progress. A closer relationship between the members of the two Societies can be of great benefit to both.

INTERACTION IN IONIC SOLUTIONS

THE Faraday Society held a general discussion on "Interaction in Ionic Solutions" at Oxford during September 17–19. The meeting was held in the University Laboratory of Physiology, and more than 180 members and visitors stayed in Balliol College, where the guest-night dinner was held. The president of the Society, Mr. R. P. Bell, in opening the discussion, reminded those present that a general discussion on "Electrolyte Solutions" had been held thirty years ago in Oxford. In fact, the president's first published contribution to the subject was presented at that meeting in 1927 in a joint paper with Sir Harold Hartley. Among others present, who had attended and contributed to the earlier discussion, was Prof. H. S. Harned (Yale), who delivered the Spiers Memorial Lecture on "Some Recent Experimental Studies of Diffusion in Liquid Systems". Prof. Harned gave an account of the elegant experimental techniques for the precision measurement of diffusion in electrolytes which have been recently developed at Yale, and indicated some of the many possible directions of further advance. The accurate determination of activity coefficients by diffusion measurements is a remarkable achievement.

The first paper to be discussed was that by Prof. H. Falkenhagen and Dr. G. Kelbg (Rostock), which dealt with the extension of the limiting-law equations. Dr. M. Eigen (Göttingen) directed attention to the information that can be obtained about the intimate details of short-range interaction in bi-bivalent electrolytes by the observations of relaxation effects in sound-absorption spectra. Dr. S. Levine and Mr. H. E. Wrigley (Manchester) reported a calculation of the interaction of two univalent ions at small separations, and considered the relevance of the macroscopic dielectric constant of the solvent in this situation. In discussion, Prof. L. Onsager (Yale) directed attention to some recent mathematical advances which might help in the electrolyte problem, while Prof. H. S. Frank (Pittsburgh) seized the opportunity to express heretical views about the Debye–Hückel theory. A paper by Prof. T. F. Young, Mr. Y. C. Wu and Dr. A. A. Krawetz (Chicago) reported some measurements of the heat effects due to interactions between ions of like charge when pairs of univalent electrolytes with an anion or cation in common are mixed.

There followed a major section devoted to the presentation and discussion of papers dealing with the incomplete-dissociation model as a way of handling short-range ion–ion interactions. Prof. C. W. Davies (Aberystwyth) surveyed the present position in an introductory paper. Prof. E. A. Guggenheim (Reading) considered the ion distribution in dilute aqueous solutions of single binary electrolytes, and concluded that although the Debye–Hückel approximation is invalid for bi-bivalent sulphates in water, the model (complete ionization to rigid spherical ions) is satisfactory if, following N. Bjerrum, ions of opposite charge approaching more closely than 10–14 A. are treated as associated. Dr. O. Redlich and Dr. G. C. Hood (Shell Development Co., California) presented the results of Raman and nuclear magnetic-resonance investigations of the dissociation of strong acids (including nitric, perchloric and sul-

phuric acids). The agreement between the results is very satisfactory. Mr. W. D. Bale, Mr. E. W. Davies, Mr. D. B. Morgans and Dr. C. B. Monk (Aberystwyth) reported on the study of various ion association equilibria by spectrophotometry. Mr. W. G. Davies, Mr. R. J. Otter and Dr. J. E. Prue (Reading) reported precise spectrophotometric results for copper sulphate solutions, and discussed the significance of the dissociation constants obtained from cryoscopic, conductimetric and spectrophotometric measurements. Dr. G. H. Nancollas (Glasgow) gave an account of attempts to obtain and interpret heat and entropy changes for ion association reactions. In the discussion of this section there was general agreement about the validity and usefulness of the incomplete dissociation model for higher-valent electrolytes, but there was some dissension, particularly between Prof. Davies and Dr. Prue, about the relation of the numerical values of the dissociation constants to the choice of a distance of closest approach of free ions.

A section on ion-solvent interaction was introduced by Prof. H. S. Frank and Dr. Wen-Yang Wen (Pittsburgh), who discussed the several ways in which they consider ions affect the structure of water. Dr. O. T. Samoilov (Moscow) had submitted a paper on "A New Approach to the Study of Hydration of Ions", but was unfortunately absent. Dr. S. R. Gupta, Dr. G. J. Hills (Imperial College) and Dr. D. J. G. Ives (Birkbeck College) considered that their recent very precise measurements of activity coefficients of aqueous hydrochloric acid solutions over a temperature-range provide evidence for changes in heat capacity which can be interpreted in terms of changing ion-solvent interactions. A theoretical paper by Dr. A. D. Buckingham (Oxford) led to the conclusion that the ion-quadrupole term is significant in the interaction between the ion and solvent molecule, and that the dielectric-saturation effect is unimportant outside the first hydration sphere.

Few real advances or firm conclusions were discernible in the ion-solvent interaction section. However, there is considerable evidence for the existence of the proton as a tetrahydrate in water. It is entirely reasonable that a hydroxonium ion forms strong hydrogen bonds with three neighbouring water molecules and Dr. K. N. Bascombe and Mr. R. P. Bell (Oxford) showed that the variation of Hammett acidity functions with concentration in concentrated strong acids can be satisfactorily interpreted with such a model. Their general conclusion was supported by Dr. Th. Ackermann's (Hamburg) study of the heat capacity and infra-red absorption spectra of aqueous acid and alkali solutions. Further support was provided by the X-ray diffraction studies of aqueous electrolyte solutions by Mr. C. L. van Panthaleon van Eck, Mr. H. Mendel and Mr. W. Boog (Royal Dutch Shell, Amsterdam). This method shows considerable promise as a direct way of exploring the solvation problem; present results suggest that water molecules in water and potassium and calcium ions are octahedrally surrounded by six water molecules.

Dr. P. A. H. Wyatt (Sheffield) considered the interpretation of acidity functions in concentrated acids. Dr. M. Kaminsky (Marburg) discussed the relationship between the dependence on temperature of viscosity of aqueous solutions and the ion-solvent interaction. A paper on the dependence on concentration of the surface potentials of a number of electrolyte solutions by Dr. J. E. B. Randles (Birmingham) suggested that the interpretation of the results is too complex to lead to reliable evidence about ion hydration. Mr. M. Smith and Dr. M. C. R. Symons (Southampton) presented a study and interpretation of the effects of changes of solvent, added electrolyte and temperature on the first electronic absorption band of the iodide ion.

J. E. PRUE

REORGANIZATION OF THE METEOROLOGICAL OFFICE

IN 1955, the Secretary of State for Air appointed a Committee under Lord Brabazon to review the organization of the Meteorological Office. This Committee took evidence from users, both inside and outside Government service, of the services provided by the Meteorological Office, and from members of the Office staff. The main features of the Committee's report, which will not be published, were given to the House of Commons in June by the Minister for Air.

The Committee agreed that the Air Ministry should continue to be responsible for the Meteorological Office, and expressed the opinions that the standing of the Office as a scientific institution is high and that users have considerable confidence in the services provided for them. In considering possible lines of advance the Committee singled out the development of numerical methods of forecasting as one of the most important, welcomed the forthcoming installation of an electronic computer in the Office, and suggested there was a possibility of achieving greater precision in local forecasting by a closer study of weather characteristics and by the use of the radar-echo method of observing cloud and precipitation.

As regards internal arrangement, the Committee recommended that the Office should be reorganized into three 'prongs' dealing, respectively, with forecasting and services, research, and administration. This reorganization was put into force in July 1957. Its recommendations also led to a regrading of the senior posts whereby the post of Director has been raised to that of Director-General, the forecasting and services 'prong' is placed under the Director of Services and research under the Director of Research, each of the two latter posts being in the grade of chief scientific officer. The administrative 'prong' now under an assistant secretary has been enlarged to make the Office more self-contained in administrative matters.

Several new posts in the grade of senior principal scientific officer have also been created, and the number of the highest posts available to members of the experimental officer class will also be increased. These measures will improve the career prospects, a matter on which the Brabazon Committee expressed considerable concern, for all classes of the staff. The need for improvement was especially evident in the scientific officer class, to which it had been difficult to attract recruits of sufficiently high ability.

On the Services side the new posts include a senior principal scientific officer to control "Techniques and

Training' with the special task of ensuring close contact between research and forecasting.

Another new post in the same grade, responsible direct to the Director-General, has been established to co-ordinate the increasingly large volume of work in international meteorological relationships.

In considering research the Brabazon Committee paid tribute to the work of the Meteorological Research Committee which, established in 1942, has had an outstanding success. The Director-General expressed in his article the gratitude of the Office to the many outside scientists who have devoted so much time to that Committee's work.

The re-organization is particularly striking on the research side, which for the first time in the history of the Office can now be said to be fully developed. The Director of Research, whose regrading has already been mentioned, will have under him a deputy-director especially responsible for research in physical meteorology. The assistant directorates of research, all at the senior principal scientific level and increased in number by two, will be respectively responsible for controlling research in dynamical meteorology—primarily in numerical forecasting—in synoptic meteorology, special operational investigations, climatological research, atmospheric physics, and instrument development. The post of head of the Meteorological Research Flight, one of the world's primary units of upper air research, has been raised to senior principal scientific officer.

The system of general supervision of the work of the Office by the Meteorological Committee has been changed. The Brabazon Committee reported that the previous Meteorological Committee, which was a large body representative of Government departments using the services of the Office, of the Royal Societies of London and Edinburgh, and the universities, had ceased to perform any very useful function. The Committee recommended the formation in its place of an advisory committee from outside Government service. The Secretary of State accepted this advice and has established a new Meteorological Committee consisting of an independent chairman, two scientists and two laymen. One of the scientists is to be the chairman of the Meteorological Research Committee and the other is to be appointed in consultation with the President of the Royal Society. The Committee will keep under review the progress, efficiency, broad policy and scale of effort and cost of the Office and ensure the maintenance of adequate contacts between the Office and users of its services. The chairman is Lord Hurcomb and the members are Sir Austin Anderson, Sir David Brunt, Sir Charles Normand and Colonel Stopford Sackville. The Meteorological Office Advisory Council for Scotland remains unchanged.

The Brabazon Committee welcomed the decision already taken to bring together the headquarter units of the Office, now situated in central London, Harrow and Dunstable, into a single group of new buildings at Bracknell. The benefits of the reorganization will be fully felt when the new buildings, of which the planning is now well advanced, come to be occupied.

ROTHAMSTED EXPERIMENTAL STATION

THE agricultural production of the British Isles, while high for the available area of land, is still capable of great expansion. Sociological considerations apart, increases in production will, to a great degree, depend on the work of agricultural research stations. Among these stations, Rothamsted is pre-eminent in the breadth of studies carried out. Its activities are, of course, not confined to the British Isles but are gradually reaching out to many parts of the world, and the Station regularly participates in work on agricultural problems of great importance to the Commonwealth.

It is impossible in the compass of a short article to do justice to, or to present a really fair picture of, the great variety of pure and applied studies being made at such a station. The following account merely mentions some lines of investigation which struck the writer as being of particular interest and which may stimulate the reader to study the report* itself.

In the Physics Department studies continue on the microclimate in crops of wheat and potatoes. In particular, data have been obtained on the condensation and evaporation regimes and a special recording anemometer has been developed for measuring low wind-speeds within the foliage. Further important fundamental studies have been on the surface physics of clays and the mechanism of soil structure-formation. The latter subject is reviewed in a special article at the end of the report.

*Report of the Rothamsted Experimental Station for 1956. Pp. 280. (Harpenden: Rothamsted Experimental Station, 1957.) 10s.

The Chemistry Department reports a large and varied programme of work. Experiments on the placement of fertilizers have continued, and the effects of different nitrogenous fertilizers, including formalized casein, have been studied on a wide range of crops. Work on the loss of nitrate by denitrification has shown that such loss is insignificant if the moisture content of the soil is less than 60–70 per cent of water-holding capacity. Studies on phosphate uptake using fertilizers labelled with phosphorus-32 show that the radiation effects of the active isotope are generally negligible.

The Pedology Department, in close collaboration with the Soil Survey, has continued to investigate the nature of soil parent materials derived from both igneous and sedimentary rocks. Important information on the formation of aggregates and the location of exchangeable cations is being obtained by calorimetric methods. Among other projects, work is continuing on water-absorption and potassium fixation by clay minerals and on the nature of the leaf extracts which affect constituents of soil profiles.

The Soil Microbiology Department is mainly concerned with the biological interactions in soil which encourage or control the growth of micro-organisms. The micro-population of the rhizosphere is, of course, specially important since it may influence the availability of nutrients and may include pathogens. A group of bacteria resistant to certain antibiotics has been found to be abundant in the root surroundings but rare elsewhere in the soil. Other important studies include the decomposition of herbicides and

insecticides in the soil and the processes of root-hair infection by nodule organisms.

Investigations in the Botany Department have shown that the higher yields of improved strains of barley and beet are not due to higher net assimilation-rates or greater leaf area but to a more effective translocation of the products of photosynthesis. It appears that the efficiency of photosynthesis cannot be significantly increased by breeding. Ecological studies of weeds have cast doubt on the efficacy of temporary leys as a means of eradicating wild oats.

The emphasis of the work of the Biochemistry Department is on the nature of enzyme action. The enzyme from bracken that destroys vitamin B has been prepared in a stable form, and its action has been studied by improved methods. Studies of tobacco mosaic virus have shown that the whole virus is not essential for infectivity, but that a low level of infectivity is associated with fragments of it. Some parts of the enzymic processes by which viruses lose their infectivity on storage in the laboratory have been examined as a preliminary to studying the mechanism of attack on susceptible hosts.

Members of the Plant Pathology Department are also studying the tobacco mosaic virus and have shown that infectivity may reside in the nucleic acid fraction and antigenic specificity in the protein. Five viruses infecting leguminous plants were identified and five more in cereals and grasses. This raises the very interesting question as to whether the beneficial effects of ploughing-up and re-seeding old leys may be partly due to the elimination of virus-infected grasses and clovers. Important work continues on sugar-beet virus and, on the mycological side of the Department, on potato blight.

The Nematology Department has paid particular attention to bud and foliar nematodes. Work is continuing, in association with the Biochemistry Department, on the hatching factors given out by potato roots, and preliminary studies have been made of the factors affecting hatching of eggs from cysts of beet eelworm and on the movement and activity of larvæ in soil.

The Insecticides Department is working on the persistence and fate of insecticides in the soil. The factors influencing the toxicity of insecticides are also receiving attention. Toxicity is, for example, influenced by temperature, and the effect of change of temperature on speed of action differs for the different insecticides. Further work has been done on the mode of action of the organo-phosphorus insecticides. It is known that they affect choline esterase, and a comprehensive study of this enzyme as it occurs in insects is being made. The effect of plant protection chemicals on beneficial insects have been further investigated and techniques devised for the diagnosis of poisoning in bees.

The Entomology Department has carried out regular surveys of Rothamsted and Woburn farms for pest insects which will be of the greatest value to those carrying out experiments in these areas. Investigations have been started of the influence of climatic factors on insects: in the field on the effect of microclimate on growth, and in the laboratory on the relation between fat metabolism and temperature. Work on the wheat bulb-fly has continued, with special attention being paid to the reaction of the larvæ to plant exudates.

In the Bee Department a very important advance has been made in the study of European foul brood. *Streptococcus pluton*, an organism suspected of causing the disease, has been isolated in pure culture, and it has been found that this organism and *Bacillus eurydice* have both to be present in a larva to cause the disease. Di-*n*-decyldimethylammonium bromide promises to be an effective control. Further studies have been made of the nature of 'queen substance'. It has been found that 'queen substance' received in regurgitated food from other workers is sufficient to inhibit development of the ovaries in the absence of a queen. It has also been shown that mammalian hormones such as androsterone, as well as extracts from queen ants, inhibit ovary development.

In the Statistics Department the electronic computor is now fully occupied, and an interesting application is the analysis of some of the very long-term experiments at Rothamsted. A useful account of the computor and its capabilities is given at the end of the report. Nation-wide surveys of fertilizer practice and cattle diseases are in progress.

Progress reports are also given by the Field Experimental Section, the farms at Rothamsted and Woburn, the Soil Survey of England and Wales and the advisor in tropical soils. The report closes with the review articles on soil structure and the electronic computor referred to above. R. M. S. PERRIN

ASTRONOMY AT THE CAPE OF GOOD HOPE

THE Report of Her Majesty's Astronomer at the Cape of Good Hope to the Secretary of the Admiralty for 1956* appears under seven headings. In the first of these, "Buildings and Grounds", reference is made to the amount of maintenance work that has been carried out, with the result that the general condition of the buildings and grounds is now very satisfactory. A new building has been erected to house the Lyot heliograph, and the new 65-h.p. fire pump, mentioned in the report for 1955, was installed in January—fortunately in time to prevent large grass fires in February and March in adjoining properties from spreading into the Observatory grounds.

Under "Instruments", accounts are given of the work carried out with the reversible transit circle, the Victoria telescope, the astrographic telescope, the 18-in. reflector, the photoheliograph, and the new d.c. amplifier (No. 6) for the astrographic photometer, which was started in 1955. The first visual observations made with the 18-in. reflector showed that a number of minor modifications were necessary, and when the report was compiled it was hoped that the instrument would be brought into routine use during the first half of 1957. A third Brown recording potentiometer has been purchased for use with the instrument.

* Report of Her Majesty's Astronomer at the Cape of Good Hope to the Secretary of the Admiralty for the year 1956. Pp. 11. (Cape of Good Hope: The Observatory, 1957.)

"Observations and Reductions" includes the routine work with the reversible transit circle, with which a total of 11,801 transits were observed during the period under review, and 530 observations were also made of the Sun, Moon, the planets and Ceres and Vesta. Of the other nine sections of this programme, reference may be made to "Proper Motions", for the determination of which 68 plates were taken with the astrographic refractor. Owing to the poor condition of the axes of the telescope, which made precise guiding of long exposures very difficult, this programme was not stressed during 1956. After the renewal of the main bearings early in 1957 every effort will be made to complete this programme as quickly as possible. Among matters of interest in "The Radcliffe Section" special mention may be made of the work of Dr. D. S. Evans, who obtained measurable spectra of five regions, in *NGC* 253, using the Newtonian spectrograph. It was possible to identify the nearer edge of this extragalactic nebula and to trace out its spiral arm system, and the spectrograms show that the system is rotating with the arms trailing. It may also be mentioned that a start has been made with the production of an atlas of southern extragalactic nebulæ: this atlas will consist of enlarged prints from selected plates, each plate being accompanied by a short description.

One disconcerting feature is mentioned under "Personal Establishment", which may not, however, be confined in its implications to the southern hemisphere. Only one Observatory officer of those recruited since the War remains on the staff, most of the others having resigned at one time or another to take up more remunerative employment. H.M. Astronomer, Dr. R. H. Stoy, adds the following comment: "This once again emphasizes the many representations that have been made to the Admiralty that the conditions and prospects at present offered are completely unattractive to potential recruits".

Under "Miscellaneous", reference is made to the regular meetings of the Cape Centre of the Astronomical Society of South Africa, and on the second and fourth Saturday evenings of each month members of the Centre have been in attendance, when the weather is fine, to show members of the public celestial objects through the 6-in. telescope. These meetings have been well attended and greatly appreciated by the public.

LINNÆUS

THE Swedish Linnean Society this year celebrated its fortieth anniversary. There are twenty or so Linnean Societies (that of London being the oldest); but the Svenska Linné-Sällskapets differs from the rest in being concerned solely with Linnæus.

The Årsskrift for 1956–57 (Vols. 39–40. Pp. 198+24. Uppsala: Almqvist and Wiksells Boktrykeri *AB.*, 1957) is admirably produced. It is surprising how, year after year, a considerable volume is issued throwing further light on aspects of Linnæus's life, writings, associations and period, and this, of necessity, with little reference to the mass of material at Burlington House in London. (Incidentally, this material still needs a good deal of sorting by someone thoroughly competent in Swedish and Latin—and able to read Linnæus's writing—such as Dr. A. Hj. Uggla, secretary and editor of the Swedish Society, who has given such valuable service in this connexion.) The first paper in the present volume is "Linnæus and Cornwall" by Spencer Savage. Its title might lead one to think that Linnæus had contrived to visit the county during his short time in England in 1736, but it refers to his interest in Cornish mineralogy. W. Borlase, vicar of Ludgvan, well known for his "The Natural History of Cornwall", was asked by his friend, John Andrew, then studying medicine at Leyden, to send a "little box" of various ores and mundics apparently to gratify the curiosity of Boerhaave. The minerals sent were shown to Linnæus who, in the opinion of Boerhaave, "understands the nature of all kinds of mettals the best of any one in the World". Gronovius undertook their naming and sent a list following Linnæus's nomenclature. A further collection was forwarded, and, with their names, Gronovius sent a copy of a report he had received from Linnæus on the liquefaction of copper. (A third set of minerals was sent, after Linnæus had left Leyden, but no copy of this was found in Borlase's papers at Penzance and Truro.)

A. J. Boerman writes about Linnæus's candidature for his medical doctorate. To save both time and money, many Swedish medical students took their degree at the University of Harderwijk previous to hearing Boerhaave's lectures at Leyden. Before presenting and defending his thesis Linnæus had to show that his general medical knowledge was of the standing expected of a doctor. It is known that he had written his thesis before leaving Sweden; but the day after arriving at Harderwijk he had to prepare a manuscript for his Candidatus Medicinae. The text of what appears to be a fair copy of the Latin original (Linnean Society, London) is given and its English translation. There is certainly much still to be learned about Linnæus's activities in Holland.

The remaining eight principal papers are in Swedish. Carl-Axel Torin writes on Linnæus as 'rusthållare' in the horseguards. At that time landlords, as part of their tenure, had to procure and maintain a number of soldiers for the standing army, and provide them with a small plot of land. The archives of the regiment show that when Linnæus acquired Hammarby in 1758 he was responsible for four dragoons and their horses. During the Seven Years War two of these dragoons lost their lives and Linnæus had much difficulty in replacing them.

A long contribution by Gunnel Hazelius-Berg and Sigurd Wallin deals with Linnæus's wardrobe at Hammarby, with much contemporary allusion. It is amusing to find that the three-cornered hat, covered with pale green silk with a rose-coloured band, so often figured as his Dutch doctor's hat, is simply one of the kind received by the successful candidates for the medical doctorate at Upsala.

Nils Sylvén gives a catalogue of the plants of Linnæus's native parish, collating his data with those given by Linnæus, and Assar M. Lindberg, recording the tradition that three ecclesiastical families in Småland—Lindelius, Tiliander and Linnæus—took

their names from a large lime tree at Jonsboda, shows that both Linnæus's father, and Tiliander, the father's uncle, so derived their names, but not Lindelius. The tree disappeared about 1823, but its site is still pointed out. Olaf Svanberg writes on "Pan Svecicus". Linnæus, in his Dalecarian journey in 1734, noticed how horses avoided certain plants. This prompted a study of which plants were eaten by various domestic animals, and twenty years later he formed a team of about thirty students to make observations; in 1749 he published a list of 600 species ("Pan Svecicus"). Prof. Svanberg has carried out an investigation on similar lines, and has established that lymphadenosis, a disease among cattle in one district of Sweden, is caused by calves eating *Ranunculus acris* which is now left standing in the fields.

Hugh Osvald discusses Linnæus's 'hay-seed'. During practically the whole of the eighteenth century Swedish agriculturists suggested the introduction of foreign plants as a means of improving fodder. Linnæus stated that a Swedish plant, *Medicago falcata*, would serve this purpose; but, in spite of his prestige, his suggestion was not generally accepted. Recently this plant has been crossed with other species of the same genus with the object of producing a fodder suitable to Swedish conditions.

A. Hj. Uggla publishes a series of letters from the younger Linnæus to Abraham Bäck, his father's most intimate friend, written in 1778, the year after his death. They contain the son's answers to questions put while Bäck was writing his commemorative address for the Academy of Science. They are of great value in throwing light on Linnæus's character and ideas and also give an impression of the difficulties the son had in following his father as professor, without his standing.

A number of smaller papers on points concerning Linnæus and the Society add to the picture of lively interest.

There is an English summary of the principal contents. A most valuable supplement is an index to the content of the forty volumes of the yearbook, which contains a wealth of information that will prove invaluable when a reasoned account of Linnæus as a scientist, his influence on the development of ideas, and his personality is attempted.

J. RAMSBOTTOM

APPLIED PROBABILITY

"APPLIED Probability" is an exceedingly valuable collection of papers badly mistitled*. In no sense could the first three contributions (by Lévy, Doob and Feller) be regarded as essays in *applied* mathematics. Thus, Lévy's paper, ostensibly about Brownian motion, studies an abstraction of that phenomenon involving $(2p + 1)$-dimensional 'time', which would not be appropriate for the description of the physicists' Brownian motion even when $p = 0$. This comment is not intended to be uncomplimentary; rather the reverse. The reviewer only regrets that many who would find interest and even inspiration in this book will leave it unopened because of its unfortunate title.

To return to Lévy's paper, a fascinating and apparently very difficult problem is suggested by the series of papers by Lévy, of which this was the first to be written (though not the first to appear). Let E_n be real n-dimensional Euclidean space (or Hilbert space, when we write $n = \infty$), and let $\mathrm{d}(x,y)$ denote the distance between the points x and y in E_n ($\| x-y \|$, if $n = \infty$). Then it is known that a Gaussian random function $f(x)$ can be constructed in such a way that

$$\mathcal{E}\,(f(x) - f(y)) = 0\,; \quad \mathrm{var}\,(f(x) - f(y)) = \mathrm{d}(x,y)$$

If A is a set of points in E_n, and if the values of $f(x)$ for x in A are given, then Lévy writes $\mathcal{E}\,(\ldots\,|A)$ and $\mathrm{var}\,(\ldots\,|A)$ for the corresponding conditional means and variances of various random quantities. In particular, if S_t is the surface of a sphere centred at the origin in E_n and of radius t, and if K_t is the region outside S_t, Lévy considers the quantities

$$\mathrm{var}(f(0) \mid K_t) = c_n t \text{ and } \mathrm{var}(f(0) \mid S_t) = c_n' t$$

which plainly depend on t in the manner indicated and on the dimensionality n. When n increases, c_n and c_n' both decrease, and it is asserted that

$$\lim_{n\to\infty} c_n = c_\infty = 0\,; \quad \lim_{n\to\infty} c_n' = c_\infty' = 1 - 2^{-1/2}$$

Now $f(x)$, as a random variable having a finite variance, can be identified modulo a suitable equivalence with a point in the Hilbert space of random variables having a finite expected square; thus, in the case $n = \infty$, we can think of $f(.)$ as mapping one Hilbert space into another in such a way that

$$\| f(x) - f(y) \| = \sqrt{\ } \| x-y \|$$

The existence of such square-root mappings is well known and some of their surprising properties have been studied[1]. One of the results of Lévy which has been noted above suggests that the following geometrical relation ought to hold for square-root mappings of the kind just described: $f(0)$ lies in the closed linear variety spanned by $f(K_t)$. If this could be proved directly, much insight would be gained into the studies Lévy has made of such many-parameter 'Brownian motions'.

The papers by Doob and Feller may both be regarded as contributions to generalized potential theory. Doob's paper summarizes results which have already been reported by him in detail elsewhere[2] and which are concerned with solutions to abstract problems of the Dirichlet type directed by the following key idea: the boundary is to be approached in such a way that the instantaneous motion at any point is controlled by the harmonic measure. There is a slight link with Lévy's paper in that in some simple concrete situations the trajectories will be those of one-parameter Brownian motion. Doob's paper is again one of a long series; the latest has just appeared in the Russian probability journal *Teoriya Veroyatnostei*. Feller might be said to be

* Applied Probability. (Vol. 7 of Proceedings of Symposia in Applied Mathematics of the American Mathematical Society.) Pp. v+104. (London: McGraw-Hill Publishing Company, Ltd., 1957.) 37s. 6d.

writing about generalized discrete harmonic functions; specifically he studies the solutions to the equations

$$x_i = \sum_{j=1}^{\infty} p_{ij} x_j \quad (i = 1,2, \ldots) \qquad (1)$$

where the coefficients p_{ij} are non-negative and sum (over the suffix j) to unity for each separate i. Such *stochastic matrices* (p_{ij}) are of immense importance in modern probability theory. The structure of the set of natural solutions to the associated equations

$$y_j = \sum_{i=1}^{\infty} y_i p_{ij} \quad (j = 1,2, \ldots)$$

(that is, the solutions such that $\Sigma\,|\,y_i\,| < \infty$) is relatively simple and has been known for some time, but the structure of the set of natural (in this case, bounded) solutions to (1) is exceedingly intricate and is just now being unravelled by Blackwell[3] and Feller. Feller studies among other things the extremal solutions to (1) and their relation to the various possible 'escape routes' for systems the stochastic motion of which is governed Markov-chainwise by the matrix (p_{ij}). It has been customary in the past to speak of the system's escape to 'infinity' if the (Cesàro) limits of the n-step transition probabilities do not total j-wise to unity. This use of language should imply that the set of states $(1, 2, 3, \ldots)$ has been compactified by the addition of a single 'point at infinity'. The main content of Feller's work shows how much more fruitful it is to adopt a more discriminating compactification, so that (so far as possible) distinct escape routes shall be provided with distinct destinations on the boundary. Feller's boundary is constructed in terms of maximal ideals in the lattice of non-negative solutions to (1). Vast new perspectives in the theory of Markov chains and processes are opened up by this work, which is a summary of a longer paper already published elsewhere[4].

The papers by Hopf and Batchelor at last make contact with the real world, the subject-matter of both being the statistical theory of turbulence. Hopf discusses this with the aid of the characteristic functional, a technique introduced by himself in turbulence theory and independently and in other contexts by Le Cam, Bochner and others. Batchelor's object is to point out that a mathematical model of turbulence is inadequate if it leads to a spectrum analytic near zero wave-number, the essential non-analyticity being associated with requirements imposed by the Navier–Stokes equation.

Münch writes on stochastic processes in astronomy. Consider, for example, an infinite galaxy containing a uniform distribution of emissive material and superpose on this a Poisson distribution of absorbing clouds all having the transmission factor q $(0 < q < 1)$. Then with suitable units the intensity observed in a particular direction will be

$$U = u_0 + qu_1 + q^2u_2 + q^3u_3 + \ldots \qquad (2)$$

where the u's are independent random variables having the distribution $\exp(-u)\,du$ $(0 < u < \infty)$. Write $F(V) = \text{prob}\ (U \leqslant V)$. One of the simpler problems discussed by Münch is the determination of $F(V)$. One simple method of procedure would be as follows: if $s > 0$ then the Laplace transform $\varphi(s)$ of the distribution of U will be

$$\varphi(s) = \mathcal{E}\{\exp(-sU)\} = \prod_{r=0}^{\infty} (1 + q^r s)^{-1}$$

$$= \sum_{m=0}^{\infty} A_m (1 + q^m s)^{-1}$$

say. Now

$$\varphi(s) = \varphi(qs)/(1 + s)$$

and on manipulating the partial fractions we easily find that

$$A_m = -q^m (1 - q^m)^{-1} A_{m-1} \quad (m = 1, 2, 3, \ldots)$$

so that

$$F(V) = C \sum_{m=0}^{\infty} (-)^m \Big(\prod_{n=1}^{m} q^n/(1 - q^n) \Big) (1 - \exp(-V/q^m))$$

where C is a normalizing constant. When a finite galaxy is considered, or when the transmission factor q is allowed to be a random variable, the problem is more difficult.

In the next paper Kac surveys equilibrium problems in statistical physics, and problems concerning the approach to equilibrium. In Part 1 he discusses the Ising model, the spherical model and the problem of the imperfect gas. In Part 2 he summarizes his "Foundations of Kinetic Theory" (ref. 5). There is much to interest the analyst in these apparently 'applied' papers.

Ulam's and Koopman's contributions are apparently less mathematical in content, being largely written in prose. Koopman discusses the foundations of probability theory in relation to quantum theory; Ulam reports on a number of studies he has made with John Pasta of mathematical 'models' rather different from those commonly discussed in physics but which, he thinks (and the reviewer agrees), may be found useful in the future. For example, consider the lattice of points with integer co-ordinates in n dimensions $(1 \leqslant n \leqslant 3)$, and assign to each lattice-point mass one or mass zero, with equal probability, the assignments to be made independently. Now rub out the construction lines (the lattice), think of the array of masses as lying in E_n and let them attract according to the law of the inverse square. It is stated that the resultant force can be well defined for all $t \geqslant 0$, and that the motion for all $t \geqslant 0$ can be studied if collisions are handled by assuming coalescence and conservation of mass and momentum. When $n = 1$, the author asserts that (i) the masses appearing in the course of time are unbounded, (ii) some particles will always remain single, (iii) the asymptotic density remains constant, and (iv) arbitrarily large 'holes' will appear in the system. The mathematics of the problem is vastly more difficult when $n = 2$ or 3, and here the author and his collaborator had to resort to Monte Carlo techniques. The results quoted are very instructive, and one looks forward to reading a more detailed account of this work. One is reminded of the balance between diffusion and accretion which is sometimes indicated (for example, by Jeans[6]) as a mechanism for stellar evolution. A detailed handling of this astronomical problem would undoubtedly raise questions of the kind considered here.

D. G. Kendall

[1] Blumenthal, L. M., "Theory and Applications of Distance Geometry" (Oxford, 1953).

[2] Doob, J. L., Proc. Third Berkeley Symp., 2, 49 (1956).

[3] Blackwell, D., *Ann. Math. Statist.*, 26, 654 (1955). Breiman, L., *ibid.*, 28, 499 (1957).

[4] Feller, W., *Trans. American Math. Soc.*, 83, 19 (1956).

[5] Kac, M., Proc. Third Berkeley Symp., 3, 171 (1956).

[6] Jeans, J. H., "Astronomy and Cosmogony", pp. 337–342 (Cambridge, 1928).

USE OF FARADAIC RECTIFICATION FOR THE STUDY OF RAPID ELECTRODE PROCESSES

By Dr. G. C. BARKER, R. L. FAIRCLOTH and A. W. GARDNER

Chemistry Division, Atomic Energy Research Establishment, Harwell

SEVERAL workers[1,2] have observed a small change in mean potential when an electrode is polarized by a sinusoidal alternating current. This phenomenon, which has been aptly termed faradaic rectification[2], is caused by the non-linearity of electrode processes as regards the variations of their rates with potential. Theoretical treatments have been given by Doss and Agarwal[3,4] and Oldham[2] for an electrode in contact with a solution containing a simple redox couple. The former workers considered only the case in which the concentrations and diffusion coefficients of the reactants are equal and found that the mean potential change for constant alternating component of potential approached a limiting value as the frequency increased. This limiting value was proportional to $1-2\alpha$, where α is the transfer coefficient of the forward process of the electrode reaction. Oldham, however, found that the potential change approached zero for all values of α as the frequency increased. Some time ago we had occasion to consider the same system. For the redox reaction

$$O + n\varepsilon \rightleftharpoons R$$

in the most general case ($C_0 \neq C_r$, $D_0 \neq D_r$) it was found that the potential change ψ is given by

$$\Psi_{(V \ll RT/nF)} = V^2 \frac{nF}{RT}\left[\frac{2\alpha-1}{4} + \left(\frac{(1-\alpha)D_0^{1/2}C_0 - \alpha D_r^{1/2}C_r}{2D_r^{1/2}C_r + 2D_0^{1/2}C_0}\right)\left(\frac{Zk_e + 2^{1/2}Z^2k_e^2}{2^{1/2} + 2Zk_e + 2^{1/2}Z^2k_e^2}\right)\right] \quad (1)$$

where $Z = \dfrac{D_r^{1/2}C_r + D_0^{1/2}C_0}{C_r\omega^{1/2}D_r^{1/2}D_0^{1/2}}$

In (1) C_0^* and C_r are the concentrations of O and R, respectively, in the bulk of the solution. D_0 and D_r are the diffusion coefficients of the two reactants. V is the amplitude of the (almost) sinusoidal component of the interfacial potential and ω is the angular frequency. The rate constant at equilibrium of the forward process of the reaction (the reduction of O) is denoted by k_e.

Equation (1) applies equally well to a liquid metal electrode when R is dispersed throughout the metallic phase. It is only valid for values of V much smaller than RT/nF, and hence, in any accurate test of (1), ψ is likely to be quite small. If $C_0 = C_r$ and $D_0 = D_r$, (1) reduces to an equation derived earlier by Doss and Agarwal[4].

The corresponding expression for ψ in the case of an electrode reaction in which the concentration of R at the electrode surface remains constant (for example, $Hg_2^{2+} + 2\varepsilon \rightleftharpoons 2Hg$) is obtained from (1) by letting $C_0/C_r \to 0$. One then obtains

$$\Psi_{(V \ll RT/nF)} = \frac{V^2nF}{4RT}\left[2\alpha - 1 - \frac{2\alpha\ (2^{1/2}k_e^2 + k_e\omega^{1/2}D_0^{1/2})}{2^{1/2}k_e^2 + 2k_e\omega^{1/2}D_0^{1/2} + 2^{1/2}\omega D_0}\right] \quad (2)$$

For both types of reaction ψ tends towards the limiting value $(2\alpha-1)V^2nF/4RT$ as ω increases, thus making possible a direct determination of the transfer coefficient if V is known. Further, by studying the variation of ψ with frequency, k_e can also be determined, if the diffusion coefficients which appear in the appropriate equation are known.

The validity of (1) has been tested by studying the variation with frequency of the amplitude of the low-frequency alternating current (the faradaic rectification or F–R current) which has to be supplied to a mercury-drop electrode to keep its mean potential constant, when it is polarized by an amplitude-modulated radio-frequency current. Square wave modulation (225 c./s.) has been used, and the F–R current has been measured with a 225 c./s. square wave polarograph[5] using the basic circuit shown in Fig. 1. Frequencies between 100 kc./s. and 6·4 Mc./s. have generally been used.

In all cases in which the kinetics have been such that the reaction at the highest frequency was almost entirely kinetically controlled, the amplitude of the F–R current for constant C_0, C_r and V has approached a limiting value, showing that ψ must also have a finite limiting value. As predicted by theory, this

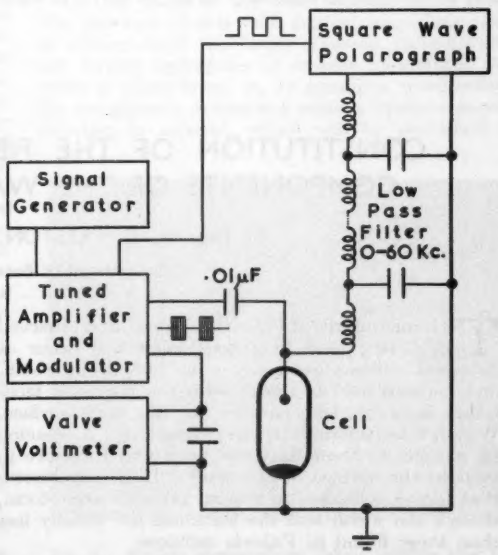


Fig. 1. Basic circuit for measurement of faradaic-rectification current

Table 1. Approximate Values of α and k_o

Medium	Temp. (°C.)	Reaction	α	k_o (cm. sec.$^{-1}$)
0·5 *M* K_2SO_4	22	$Cd^{2+} + 2e \rightleftharpoons Cd(Hg)$	0·08	0·08
		$Tl^+ + e \rightleftharpoons Tl(Hg)$	0·68	3·1
1 *M* $NaClO_4$ (*p*H 2)*	22	$Pb^{2+} + 2e \rightleftharpoons Pb(Hg)$	0·60	3·3
		$Cd^{2+} + 2e \rightleftharpoons Cd(Hg)$	0·07	2·3
		$Tl^+ + e \rightleftharpoons Tl(Hg)$	0·69	1·2
1 *M* KNO_3 (*p*H 2)*	22	$Pb^{2+} + 2e \rightleftharpoons Pb(Hg)$	0·81	0·8
		$Cd^{2+} + 2e \rightleftharpoons Cd(Hg)$	0·15	6·3
		$Tl^+ + e \rightleftharpoons Tl(Hg)$	0·8	0·3
1 *M* KCl (*p*H 2)*	22	$Pb^{2+} + 2e \rightleftharpoons Pb(Hg)$	0·94	0·2
		$Cd^{2+} + 2e \rightleftharpoons Cd(Hg)$	0·78	2·9
		$Tl^+ + e \rightleftharpoons Tl(Hg)$	0·9	0·15
Ternary eutectic $LiNO_3$, $NaNO_3$, KNO_3 + 0·02 mole per cent KCl	156	$Pb^{2+} + 2e \rightleftharpoons Pb(Hg)$	0·83	1·4
		$Cd^{2+} + 2e \rightleftharpoons Cd(Hg)$	0·60	0·53

* *p*H adjusted by addition of perchloric acid.

value is independent of C_0 and C_r and its sign is governed by the sign of $1 - 2\alpha$.

The kinetics of various rapid electrode reactions have been investigated by studying the dependence of the *F–R* current on ω and on the concentration ratio C_0/C_r, using values of C_0 and C_r smaller than $\frac{2}{n^2} \times 10^{-4}$ *M* and values of V smaller than 5 mV.

Some approximate values of α and of k_o, the rate constant of the forward process of the reaction when the rate constants of the forward and reverse processes are equal, are given in Table 1. For the reduction of cadmium ion in 0·5 *M* sulphate solution the values of α and k_o are in fair agreement with values deduced from data given by Gerischer[6]. For the reduction of the same ion in molar potassium nitrate solution, k_o is about ten times larger than a rate constant (approximately equal to k_o) reported by Randles and Somerton[7]. In no other cases has it been possible to compare the present results with results obtained by other workers. Table 1 shows that in some cases α is surprisingly small ($\sim 0{\cdot}07$) and in others it is unusually large ($\sim 0{\cdot}9$). In such instances the main rate-controlling step in the reduction of the ion may possibly be a chemical process. For example, slow dehydration of the ion prior to its transfer to the electrode may be responsible for values close to zero, while slow formation of a soluble intermetallic compound between the metal and mercury could lead to an apparent value of α close to unity. However, it is not easy to see why the latter process should tend to become the rate-determining process in the case of chloride solutions, and it seems more likely that the large values of α in some way are connected with the reduction of complex ions.

The faradaic rectification method appears to be a powerful method for the study of very rapid electrode processes. It is free from errors associated with the resistance of the solution such as occur when conventional methods[8] are employed. Also, if C_0 and C_r are small the interfacial impedance at radio-frequencies is controlled by the double-layer capacity, and, provided that this capacity is an aperiodic quantity, errors connected with its low impedance do not arise. To avoid systematic errors, small corrections must be applied to allow for the effect of the slight non-linearity of the double-layer capacity, and for the effect of changes with frequency in the distribution of the polarizing current over the electrode surface. At the present time, uncertainties in the magnitude of the latter correction and in the value of D_0 are often the major sources of error in the determination of α and k_o.

[1] Doss, K. S., and Agarwal, H. P., *Proc. Indian Acad. Sci.*, **34**, 229 (1951). Hillson, P. J., *Trans. Faraday Soc.*, **50**, 385 (1954).
[2] Oldham, K. B., *Trans. Faraday Soc.*, **53**, 80 (1957).
[3] Doss, K. S., and Agarwal, H. P., *Proc. Indian Acad. Sci.*, **34**, 263 (1951).
[4] Doss, K. S., and Agarwal, H. P., *Proc. Indian Acad. Sci.*, **35**, 45 (1952).
[5] Barker, G. C., and Jenkins, I. L., *Analyst*, **77**, 685 (1952).
[6] Gerischer, H., *Z. Elektrochem.*, **57**, 604 (1953); *Z. physik. Chem.* (N.F.), **10**, 264 (1957).
[7] Randles, J. E. B., and Somerton, K. W., *Trans. Faraday Soc.*, **48**, 937 (1952).
[8] Delahay, P., "New Instrumental Methods in Electrochemistry" (Interscience Publishers, New York, 1954).

CONSTITUTION OF THE FIBRILLAR AND NON-FIBRILLAR COMPONENTS OF THE WALLS OF *VALONIA VENTRICOSA*

By Prof. R. D. PRESTON, F.R.S., and J. CRONSHAW

Department of Botany, University of Leeds

THE microfibrils of *Valonia* cellulose, first observed in 1948[1], have long been known to occur as flattened ribbons averaging some 200 A. in width, and perhaps half as thick[2], with the flattened faces lying more or less parallel to the wall surface. Within these microfibrils the planes of 6·1 A. spacing lie parallel to these flattened faces and therefore in turn to the surface of the wall[3]. It is now known that native celluloses in general take the same form, though the width and the thickness are usually less than those found in *Valonia* cellulose.

Various lines of evidence, however, have suggested that the microfibrils of *Valonia* cellulose type are unique in possessing an extraordinarily high degree of crystallinity. Thus the X-ray diagram of the untreated *Valonia* wall, and the electron diffraction diagram as observed in the electron microscope, suggest that the crystallites of this cellulose are about as broad as the microfibrils themselves[3,4]; the cellulose is soluble only with extreme difficulty in cuprammonium and a positive reaction with iodine and sulphuric acid or with chlorzinc iodide is always difficult to obtain and often lacking[5]; the wall does not swell in 72 per cent sulphuric acid[3] and the microfibrils are scarcely affected by acid of that strength. It has, however, always appeared to be a

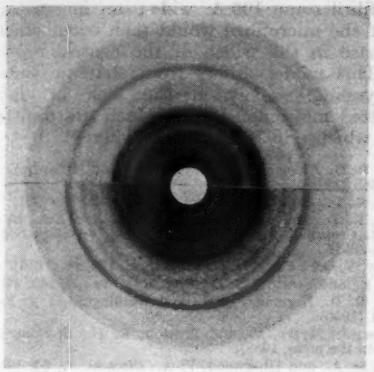


Fig. 1. X-ray powder diagrams of *Valonia* cellulose and cotton cellulose. Copper $K\alpha$ radiation collimated to a beam 0·5 mm. in diameter. Specimen-film distance, 3 cm. (*a*) a pellet of purified *Valonia* cellulose; (*b*) a pellet of purified cotton fibres

paradox that the percentage crystallinity as determined by the method of Hermans is no higher than 70 per cent[6] and therefore of the same order as that in the cellulose of many fibres in which the crystallinity is fairly low as judged by other methods.

Little is known, however, concerning the chemical constitution either of the microfibrils or of the whole wall of *Valonia*, and it is obviously essential to know something about this before the figure of 70 per cent can be made significant. During a recent investigation of the chemical constitution of celluloses from various sources[7] we have included but not reported upon *Valonia* cellulose. In this study the wall was fractionated, and the sugars in the hydrolysis products detected, by the methods of Jermyn and Isherwood[8]. In addition, the chemical extraction process described by Roelofsen and Houwink[9] was also used to obtain a preparation of the pure cellulose component. It was found that the cellulose component, observed in the electron microscope to consist entirely of microfibrils with little or no associated amorphous material, represents about 75 per cent of the wall dry weight. This therefore would seem to harmonize the results obtained by Hermans's method with the high crystallinity associated with other observations. On hydrolysis, this material yields glucose only, as shown by paper chromatography. The other wall material extracted by boiling water and by treatment with 4 *N* potassium hydroxide gave upon hydrolysis a mixture of galacturonic acid, galactose, glucose, arabinose and xylose. It is impossible to say at this time whether any of these extracted sugars were extracted from the microfibrils.

The X-ray diagram of a compacted pellet of the purified microfibrils is presented in Fig. 1*a* in comparison with a similar diagram of cotton cellulose produced under the same conditions (Fig. 1*b*). It will be clear that the prominent ring of 3·9 A. spacing (the fourth ring from the centre) is noticeably narrower than the corresponding ring in the cotton diagram. It is a little difficult to pronounce upon the relative thickness of the other rings, since the relative intensities differ, owing presumably to some trivial cause in, for example, the packing of the microfibrils. This, however, indicates without any doubt that the crystallites in *Valonia* cellulose are broader than are those in cotton, which itself is already known to be rather highly crystalline.

We suggest, therefore, that it is reasonable to consider the microfibrils of *Valonia* cellulose as very highly crystalline, consisting of a central core of crystalline cellulose measuring on an average about 200 A. × 100 A. and containing, therefore, something of the order of 600 molecular chains. These may be clothed, perhaps, in one or two layers of chain molecules the crystallinity of which is upset by the incorporation of chain compounds other than glucans; one such layer would then contain about 100 chains more randomly arranged than those of the central core.

Two further observations show that this central crystalline core does not, however, run uninterruptedly along the whole length of a microfibril, and that the microfibril is therefore heterogeneous along its length. First, treatment of the microfibrils with sulphuric acid of sufficient strength breaks down the microfibrils into shorter lengths. This supports an earlier observation[3] that when sulphuric acid begins to attack *Valonia* cellulose it does so at local regions along the microfibril. When the microfibril becomes broken through at these localities, further hydrolysis ceases. Secondly, whenever *Valonia* microfibrils are observed in an electron microscope after treatment—prolonged boiling in water is often sufficient—the effect illustrated in Fig. 2 is frequently observed. It appears that, at positions irregularly distributed along the length of a microfibril, pieces of the microfibril have been removed although the shadow produced by metal shadowing is continuous across the gap. These pieces must therefore have been removed in the electron microscope, and have presumably been so changed even by the mild treatment as to become more sensitive to bombardment by electrons. It would appear that there are regions of weakness irregularly distributed along the length of the microfibrils, in which, therefore, presumably the thickness of this central core is reduced or the crystallinity disturbed in some other way.

On the basis of the observations which indicate the presence of this solid central crystalline core, it is evident that the larger *Valonia* microfibrils are not merely aggregates of smaller microfibrils of the order of those found in, for example, wood cellulose. On the grounds of this and other evidence concerning cellulose in general, which will be published else-

Fig. 2. Electron micrograph of *Valonia* cellulose after mild treatment with hydrochloric acid (× 24,000). Palladium–gold shadowed. Note the obvious breaks in the fibre across which the shadow is nevertheless continuous

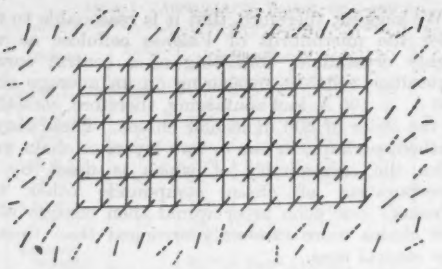


Fig. 3. Diagrammatic representation of the transverse section of a cellulose microfibril about 100 A. wide and about 50 A. thick. The solid strokes represent the planes of the glucose residues in the cellulose chain molecule and the broken strokes the planes of other sugars or sugar derivatives in non-cellulosic molecular chains. The area joined into a lattice represents the solid central core

where[10], it is suggested that the constitution of cellulose microfibrils in general is somewhat as illustrated in cross-section in Fig. 3. This figure refers to a microfibril some 100 A. wide; an increase in the width of the microfibril would then occur chiefly by an increase in the width of the central crystalline core. This model differs from that presented by Frey-Wyssling[11] solely in the lack of 'internal' randomized molecular chains which are omitted for reasons which will be given elsewhere[10].

[1] Preston, R. D., Nicolai, M. F. E., Reed, R., and Millard, A., *Nature*, **162**, 665 (1948).
[2] Preston, R. D., Faraday Society Discussion No. 11 (1951).
[3] Preston, R. D., and Astbury, W. T., *Proc. Roy. Soc.*, B, **122**, 76 (1937). Cronshaw, J., and Preston, R. D., *Proc. Roy. Soc.* (in the press, 1958).
[4] Balashov, V., and Preston, R. D., *Nature*, **176**, 64 (1955).
[5] Nicolai, E., and Preston, R. D., *Proc. Roy. Soc.*, B, **140**, 244 (1952).
[6] Preston, R. D., Hermans, P. H., and Weidinger, A., *J. Exp. Bot.*, **1**, 344 (1950).
[7] Cronshaw, J., Myers, A., and Preston, R. D., *Biochim. Biophys. Acta* (in the press, 1958).
[8] Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **64**, 123 (1956).
[9] Roelofsen, P. A., and Houwink, A. L., *Acta Botanica Neerlandica*, **2**, 218 (1953).
[10] Preston, R. D., presented to "International Review of Cytology" (1958).
[11] Frey-Wyssling, A., *Science*, **119**, 80 (1954).

INHIBITION OF CAROTENOID SYNTHESIS IN PHOTOSYNTHETIC BACTERIA

Specific Inhibition of Carotenoid Synthesis in a Photosynthetic Bacterium and its Physiological Consequences

WE have suggested[1-3] that the carotenoid pigments characteristically associated with the photosynthetic apparatus perform an essential physiological function, by protecting the cell from the deleterious effects of chlorophyll-catalysed photooxidations. The hypothesis was initially formulated as a result of observations on the behaviour of a carotenoidless mutant of the photosynthetic bacterium *Rhodopseudomonas spheroides*. The mutant in question synthesizes almost normal quantities of bacteriochlorophyll, but the coloured carotenoids characteristic of the wild type are completely absent from its cells, their place being taken by a much more reduced colourless C_{40} polyene, phytoene. The mutant can grow photosynthetically under anaerobic conditions in the light; but when photosynthetically grown cells are exposed simultaneously to light and air, they are rapidly killed and their bacteriochlorophyll is destroyed. The photosensitizing pigment is bacteriochlorophyll itself. Such photosensitivity is not manifested by the wild type, or by mutants in which the normal carotenoids have been replaced by other coloured carotenoids. The correlation between carotenoid deficiency and aerobic photosensitivity has since been demonstrated by Kandler and Schötz in a mutant of *Chlorella* with derangements of normal carotenoid synthesis[4].

Specific depletion of carotenoid pigments has been caused by gene mutation in the photosynthetic organisms so far studied. We have now found that specific depletion can be produced without a change of genotype in the photosynthetic bacterium *Rhodospirillum rubrum*, as a result of growth in the presence of diphenylamine. Under these conditions, cells of *R. rubrum* become almost devoid of coloured carotenoids, but maintain a normal content of bacteriochlorophyll. Exposed to light and air, such cells display the symptoms of photosensitivity (death and chlorophyll destruction) previously observed in the carotenoidless mutant of *Rhodopseudomonas spheroides*. In *Rhodospirillum rubrum*, carotenoid depletion is readily reversible. When the diphenylamine is removed, rapid carotenoid synthesis at the expense of accumulated endogenous precursors begins immediately. The restoration of coloured carotenoids is accompanied by a rapid diminution of the induced photosensitivity. This reversible physiological induction of photosensitivity, correlated with changes in carotenoid content, provides additional experimental support for our hypothesis concerning the function of carotenoid pigments in photosynthetic organisms.

Effects of diphenylamine on growth and pigment synthesis in Rhodospirillum rubrum. The inhibition of carotenoid synthesis by diphenylamine has been observed in many fungi and bacteria, and was first demonstrated in *R. rubrum* by Goodwin and Osman[5,6]. They reported, however, that the synthesis of bacteriochlorophyll was simultaneously suppressed, an observation which we have been wholly unable to confirm. Fig. 1 shows an experiment which illustrates the strikingly specific effect of diphenylamine on carotenoid synthesis. At the point indicated by an arrow, diphenylamine (final concentration: 7×10^{-5} *M*) was added to a culture of *R. rubrum* in the course of exponential photosynthetic growth under steady-state conditions of chlorophyll and carotenoid synthesis. The synthesis of coloured carotenoids ceased immediately, whereas both growth and chlorophyll synthesis continued, though at a lower rate. The parallelism of the curves for growth and chlorophyll synthesis shows that the differential rate of chlorophyll synthesis remained essentially unchanged. The extent to which the chlorophyll:

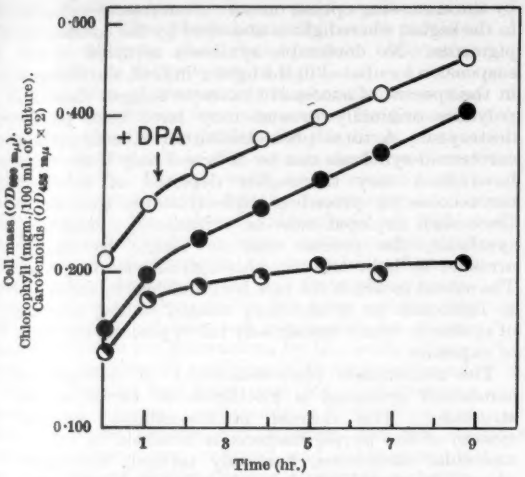


Fig. 1. Effect of diphenylamine (7×10^{-4} *M*) on growth and pigment synthesis by a culture of *Rhodospirillum rubrum*, developing exponentially in anaerobiosis and light. Pigments were measured in acetone–methanol extracts of the cells. Values for chlorophyll were calculated from the optical density of the extract at 775 mμ. Values for the coloured carotenoids are expressed in arbitrary terms, as the optical density of the extract at 485 mμ (the absorption maximum of the mixed carotenoids), corrected for light absorption at this wave-length by chlorophyll. ○—○, cell mass; ●—●, chlorophyll; ◑—◑, coloured carotenoids

carotenoid ratio can be modified by the cultivation of *R. rubrum* in the presence of diphenylamine is evident from the absorption spectra of normal and treated cells, grown photosynthetically under otherwise identical conditions (Fig. 2).

The suppression of normal carotenoid synthesis provoked by diphenylamine is accompanied by a massive intracellular accumulation of the more saturated polyenes, which are undetectable in normal cells. The major component is phytoene, accompanied by smaller quantities of phytofluene, ζ-carotene and neurosporene. Indications of this accumulation can be seen in the spectrum of the cells (Fig. 2). In treated cells, the bacteriochlorophyll peak at 375 mμ is much higher relative to the infra-red peak at 880 mμ than in normal cells. This is caused by the light-absorption of saturated polyenes in the near ultra-violet region. Quantitative analyses of the extracted polyenes, kindly performed for us by Dr. T. Nakayama, showed that the total polyene content of cells grown in the presence of diphenylamine is far higher than the content of carotenoid pigments in normal cells. In this respect as well, the findings of Goodwin and Osman[6], who detected only 'traces' of the more saturated polyenes in cells of *R. rubrum* grown with diphenylamine, could not be confirmed.

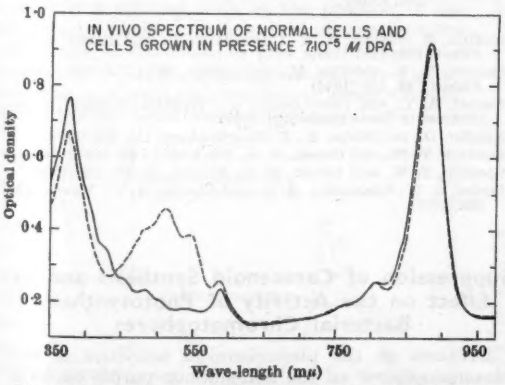


Fig. 2. The absorption spectra of two suspensions of *Rhodospirillum rubrum* grown anaerobically in the light and adjusted to the same optical density at 680 mμ before measurements were made. Spectra were determined through opal glass to reduce the effect of light-scattering. Dashed line: normal cells. Solid line, cells grown in the presence of 7×10^{-5} *M* diphenylamine

As discovered by Goodwin and Osman[6], an extensive and rapid synthesis of coloured carotenoids can occur when cells which have been grown in the presence of diphenylamine are washed and resuspended in buffer. This endogenous synthesis proceeds with equal rapidity under aerobic conditions in the dark and under anaerobic conditions in the light, but cannot occur under anaerobic conditions in the dark. It is presumably an energy-requiring process, for which energy can be supplied either by photophosphorylation in the light, or by oxidative phosphorylation in the dark. Since Goodwin and Osman could detect only traces of saturated polyenes in cells treated with diphenylamine, they assumed that the endogenous synthesis occurred at the expense of non-specific intracellular precursors. We have re-studied this question in collaboration with Mrs. Synnøve Jensen and Dr. T. Nakayama. The coloured carotenoids are in fact synthesized *exclusively* from the accumulated polyenes, through a succession of intermediates. These findings and their biochemical implications will be described elsewhere.

Induced photosensitization of Rhodospirillum rubrum. Representative results of two experiments which demonstrate the photosensitivity induced in *R. rubrum* by carotenoid depletion are given in Table 1. Cells harvested after growth in the presence of diphenylamine were washed and resuspended in chilled buffer. Aliquots of this suspension were added to aeration tubes, which were placed in a water-bath held at 30° C. and aerated with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide in the dark, to prevent any occurrence of endogenous carotenoid synthesis. At the start of experiments, the aeration mixture was changed to 95 per cent air and 5 per cent carbon dioxide. Illuminated tubes were exposed to a light intensity of approximately 1,000 foot-candles from incandescent lamps, and dark control tubes were completely shielded by a wrapping of aluminium foil. Samples were removed at intervals for determinations of viable count and chlorophyll content. As shown in Table 1, photo-killing is relatively rapid: in the presence of light and air, the viable count fell to less than 20 per cent of its original value within 2 hr. The viable counts of the dark

Table 1. Photo-killing and Chlorophyll Destruction in Buffered Suspensions of Cells, depleted of Coloured Carotenoids by Growth in the presence of Diphenylamine

		Conditions of exposure		
		Air, light	Air, dark	Air, light, after 30 min. in air, dark
Exp. 1	Per cent survivors after 2 hr.	14	87·5	—
	Per cent chlorophyll after 23 hr.	39	106	
Exp. 2	Per cent survivors after 1·5 hr.	19·4	97	55·5
	Per cent chlorophyll after 23 hr.	75	100	92

controls also fell slightly, but this decline was comparatively negligible. The photo-destruction of chlorophyll is far slower than photo-killing, and much longer exposures are accordingly required for its accurate measurement. As shown in Table 1, the chlorophyll content of illuminated suspensions had declined markedly after 23 hr., whereas that of the dark controls was essentially unchanged. Comparable exposures to air and light cause no measurable death or chlorophyll destruction in normal cells.

Exp. 2 in Table 1 also illustrates the diminution of photosensitivity which occurs when cells are permitted to initiate endogenous synthesis of carotenoid pigments before exposure to light. In this experiment, an aliquot of the cell suspension was kept for 30 min. under aerobic conditions in the dark before exposure to light. The moment of light exposure was used as the zero time for measurements of viability and chlorophyll content on this tube. It can be seen that both death and chlorophyll destruction were much less rapid than in the suspension which was exposed to light and air without preceding maintenance under aerobic conditions in the dark. Desensitization was not complete; but the amount of endogenous carotenoid synthesis which had taken place at the time of light exposure was very limited. Complete restoration of the normal coloured carotenoids requires many hours.

Photo-inhibition of endogenous carotenoid synthesis. In *R. rubrum*, the photosensitivity which accompanies carotenoid depletion has one additional manifestation: inhibition of endogenous carotenoid synthesis. Fig. 3 shows the absorption spectra of acetone–methanol extracts prepared at successive intervals from two tubes of a buffered cell suspension incubated aerobically at 30° C. in the light and in the dark, respectively. Vigorous endogenous synthesis occurred in the suspension incubated in the dark, as evidenced by the increasing optical density of successive extracts in the region where light is absorbed by the carotenoid pigments. No detectable synthesis occurred in the suspension incubated in the light; in fact, the changes in the spectra of successive extracts suggest that the polyenes originally present may have been partly destroyed. A total photo-inhibition of endogenous carotenoid synthesis can be obtained only if the cells have been very thoroughly depleted of coloured carotenoids by preceding diphenylamine treatment. Once such depleted cells have initiated endogenous synthesis, the process can no longer be wholly arrested by light and air, although its rate may fall. The extent to which the rate is diminished by exposure to light and air is inversely related to the amount of synthesis which has already taken place at the time of exposure.

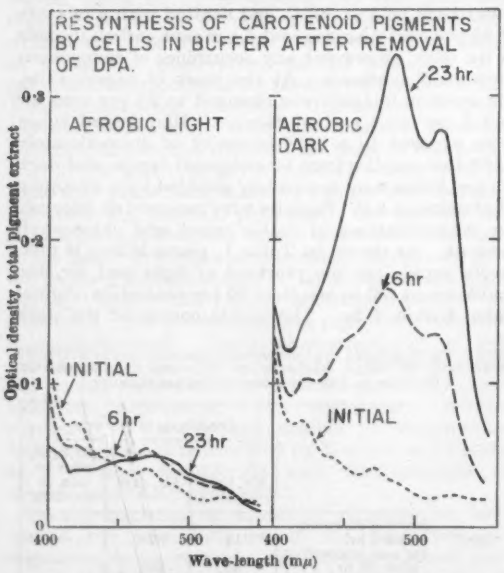


Fig. 3. The absorption spectra of acetone–methanol extracts prepared from two aliquots of a buffered suspension of cells which had been grown in the presence of diphenylamine. The suspensions were incubated aerobically at 30° C. in the light and in the dark, respectively, and samples were withdrawn after the times indicated for the preparation of extracts

The remarkable photosensitivity of endogenous carotenoid synthesis is intelligible in terms of cell structure. The normal photosynthetic pigment system of the purple bacteria is localized in macromolecular structures, variously termed 'chromatophores' or 'grana', which can be separated from other cellular components in extracts of the bacteria by differential centrifugation[7]. These bodies are the functional equivalents of plant chloroplasts, although they are much smaller and appear to be much simpler. By the fractionation of bacterial extracts, we have found that the polyenes which accumulate as a result of the diphenylamine treatment are almost exclusively associated with the chromatophores. This implies that the terminal steps of carotenoid synthesis, which take place at the expense of the saturated polyenes when diphenylamine is removed, occur in the chromatophores, where the saturated polyenes presumably fill the sites occupied by coloured carotenoids in normal cells. The substrates (and possibly also the enzymes) for endogenous carotenoid synthesis are therefore situated in the cell very close to the bacteriochlorophyll, and as a result are highly exposed to chlorophyll-catalysed photo-oxidation.

This work was supported by a grant from the National Science Foundation.

Germaine Cohen-Bazire
R. Y. Stanier

Department of Bacteriology,
University of California,
Berkeley.

[1] Griffiths, M., Sistrom, W. R., Cohen-Bazire, G., and Stanier, R. Y., *Nature*, **176**, 1121 (1955).
[2] Sistrom, W. R., Griffiths, M., and Stanier, R. Y., *J. Cell. Comp. Physiol.*, **48**, 459 (1956).
[3] Stanier, R. Y., and Cohen-Bazire, G., "Microbial Ecology", p. 56 (University Press, Cambridge, 1957).
[4] Kandler, O., and Schötz, F., *Z. Naturforschung*, **11b**, 708 (1956).
[5] Goodwin, T. W., and Osman, H. G., *Biochem. J.*, **53**, 541 (1953).
[6] Goodwin, T. W., and Osman, H. G., *Biochem. J.*, **56**, 222 (1954).
[7] Pardee, A. B., Schachman, H. K., and Stanier, R. Y., *Nature*, **169** 822 (1952).

Suppression of Carotenoid Synthesis and its Effect on the Activity of Photosynthetic Bacterial Chromatophores

Studies on the photochemical activities of the chromatophores[1] of the non-sulphur purple bacteria have demonstrated that these particles can carry out the light-dependent formation of adenosine triphosphate[2]. We have recently reported on the photochemical activities of highly purified chromato-

phores of the photosynthetic sulphur bacterium, *Chromatium*[3]. The photochemically active particles of these obligate anaerobes produce adenosine triphosphate in the presence of light.

The function of carotenoids in photosynthesis has been an open question for many years. Evidence has been offered in some organisms for the direct participation of carotenoids in the capture and transfer of light energy[4]. Recent work has indicated that carotenoids are essential for the functioning of the 'Hill reaction' by isolated chloroplasts of higher plants[5]. Stanier[6] and co-workers have demonstrated in the non-sulphur purple photosynthetic bacteria that carotenoids protect the cell from photo-oxidations by bacteriochlorophyll. We have investigated the relationship of coloured carotenoids to photophosphorylation in *Chromatium*, by the study of suppression of carotenoid synthesis with diphenylamine.

Diphenylamine has been used for inhibiting the synthesis of coloured carotenoids in fungi, algæ and photosynthetic bacteria by Goodwin and his co-workers[7]. *Chromatium*, cultured and subcultured in the presence of diphenylamine, becomes a bright blue-green in colour and lacks any visible red carotenoids. The organisms grew readily in the presence of light with carbon dioxide as the only source of carbon, and there was no apparent effect on the total amount of bacteriochlorophyll produced. 'Carotenoidless' cells regain their coloured carotenoids in the absence of diphenylamine. Red 'Cellophane' filters were used between the light source and the cultures to reduce the destruction of diphenylamine by the shorter wave-lengths of light, and under these conditions the cells remained green during the growth period (3–4 days).

Chromatophores were isolated and concentrated as previously described[3] from both the normal and the diphenylamine-treated ('carotenoidless') bacteria, and the particles sedimenting at 144,000 *g* were washed with water before use in the following experiments. The absorption spectra of the chromatophores from both types of cells are shown in Fig. 1. As can be seen, the major coloured carotenoid peaks between 450 and 550 mμ have almost completely disappeared and there is at least one new peak at 420 mμ which probably represents a polyene precursor of the coloured carotenoids. It can be seen that the major near infra-red peak of bacteriochlorophyll is unaffected but there is a striking shift in the position of the various minor peaks. The normal chromatophores of our strain of *Chromatium* give an indication of absorption at 807, 825, 840 and 895 mμ (see Fig. 2). In the absence of coloured carotenoids (green chromatophores) the 807 peak remains unchanged and there

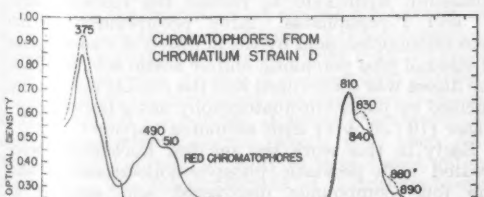


Fig. 1. Absorption spectra of chromatophores of normal and diphenylamine-grown *Chromatium*

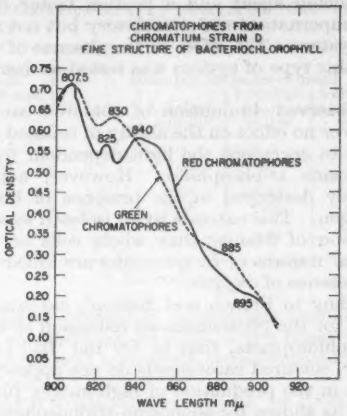


Fig. 2. Fine structure of bacteriochlorophyll absorption band for normal and diphenylamine-bleached chromatophores

is a peak at 830 and a shoulder at 885 mμ. Stanier shows a shift of the near infra-red peaks in his blue-green mutant of *Rhodopseudomonas spheroides* to a symmetrical band of the *R. rubrum* type. Duysens[8] has suggested that the various shapes of the multiple peaks of the bacteriochlorophyll absorption spectrum in different species may be due to a difference in the relationship of bacteriochlorophyll to various 'bearer molecules'. Stanier proposes that for his mutant of *R. spheroides* the shift is due to the substitution of the polyene precursors for the coloured carotenoids in the structural composition of the chromatophores. This hypothesis is extremely attractive for the diphenylamine-altered chromatophores of *Chromatium*, since similar shifts in the red spectrum are observed and the presence of precursors is indicated.

Photosensitization of photophosphorylation. As previously reported, the chromatophores of *Chromatium* will make adenosine triphosphate in the presence of light and in the absence of oxygen at a rate up to 70 micromoles of phosphate esterified per mgm. bacteriochlorophyll per hour (ref. 3). Catalytic amounts

Table 1. PHOTOSENSITIVITY OF PHOTOPHOSPHORYLATION

Pre-treatment of chromatophores*		Type of chromatophore	
Illumination (min.)	Gas phase	'Carotenoidless'	Normal
		Per cent activity†	
10 (light)	Air	0	63
10 (light)	Nitrogen	72	100
10 (darkness)	Air	100	100
30 (light)	Air	0	42
30 (light)	Nitrogen	82	88
30 (darkness)	Air	100	100
30 (darkness)	Nitrogen	100	100

* Pre-treatment of chromatophores and photophosphorylation measurements were carried out in an illuminated Warburg apparatus with 3,000 and 1,000 ft.-candles of light, respectively, at 20° C. The reaction mixtures of 3 ml. volume contained 0·1 *M* glucose, 0·02 *M tris* buffer at *p*H 7·8, 7·5 μmoles adenosine diphosphate at *p*H 7·8, 10 μmoles magnesium chloride, 25 μmoles orthophosphate, 0·3 ml. of supernatant from ruptured cells, 4 mgm. crude hexokinase (Sigma Chemical Co.), normal or 'carotenoidless' chromatophores containing 0·25 and 0·32 mgm. of bacteriochlorophyll, respectively. Chromous chloride was present in the centre well of the anaerobic flasks. After the various pre-treatments, the vessels were flushed with nitrogen in the dark, the vents were closed, adenosine diphosphate was tipped in, and photophosphorylation was initiated by turning on the lights. Disappearance of orthophosphate was determined (ref. 9) and used as a measure of photophosphorylation.

† Normal and 'carotenoidless' chromatophores esterified, respectively, 60 and 61 μmoles of phosphate per mgm. of bacteriochlorophyll per hour. This was used as the '100 per cent activity' figure.

of a reducing agent and a protein factor from the cellular supernatant are stimulatory but not required by the system. The effect of the absence of carotenoids on this type of system was tested as described in Table 1.

The observed diminution of coloured carotenoids has little or no effect on the ability of isolated chromatophores to carry out the light-dependent formation of adenosine triphosphate. However, activity is completely destroyed in the presence of both light and oxygen. This extends to an isolated system, the observation of Stanier that whole cells of a 'carotenoidless' mutant of *R. spheroides* are killed by light in the presence of oxygen.

According to French and Bishop[5], carotenoids are essential for the photochemical reduction of a dye by isolated chloroplasts, that is, for the 'Hill reaction'. However, coloured carotenoids do not appear to play any part in the production of high-energy phosphate by light as shown by adenosine triphosphate formation with green chromatophores. Therefore it is probable that the production of high-energy phosphate by the chromatophores precedes or is independent of the photochemically induced reduction of a dye.

This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

R. C. Fuller
I. C. Anderson

Biology Department,
Brookhaven National Laboratory,
Upton, Long Island, N.Y.

[1] Schachman, H. K., Pardee, A. B., and Stanier, R. Y., *Arch. Biochem. Biophys.*, **38**, 245 (1952).
[2] Frenkel, A. W., *J. Biol. Chem.*, **222**, 823 (1956).
[3] Anderson, I. C., and Fuller, R. C., *Plant Physiol.*, **32** (supplement), xvi (1957). Anderson, I. C., and Fuller, R. C., *Arch. Biochem. Biophys.* (in the press).
[4] Blinks, L. R., "Ann. Review Plant Physiol.", **5**, 93 (1954).
[5] Lynch, V. H., and French, C. S., *Arch. Biochem. Biophys.*, **70**, 382 (1957). Bishop, N. I., *Plant Physiol.*, **32** (supplement), liv (1957).
[6] Griffiths, M., Sistrom, W. R., Cohen-Bazire, G., and Stanier, R. Y., *Nature*, **176**, 1211 (1955). Sistrom, W. R., Griffiths, M., and Stanier, R. Y., *J. Cell. Comp. Physiol.*, **48**, 459 (1956). Stanier, R. Y., and Cohen-Bazire, G., "Microbial Ecology", p. 56 (Cambridge University Press, 1957).
[7] Goodwin, T. W., and Osman, H. G., *Biochem. J.*, **53**, 541 (1953). Goodwin, T. W., and Jamikorn, M., *J. Protozool.*, **1**, 216 (1954).
[8] Duysens, L. N. M., Thesis, Univ. of Utrecht. Cited from Rabinowitch, E. I., "Photosynthesis", **2**, 1853 (New York: Interscience Publishers, Inc., 1956).
[9] Lowry, O., and Lopez, J., *J. Biol. Chem.*, **162**, 421 (1946).

NATURAL OCCURRENCE OF THYMINE AND THREE METHYLATED ADENINE BASES IN SEVERAL RIBONUCLEIC ACIDS

By Dr. J. W. LITTLEFIELD* and Dr. D. B. DUNN

Agricultural Research Council Virus Research Unit, Molteno Institute, Cambridge

ALTHOUGH three naturally occurring bases in addition to adenine, guanine, cytosine and thymine have been isolated from deoxyribonucleic acid[1,2], the compound studied by Cohn, Davis and Allen[3] seems the only example so far of any additional bases naturally present in ribonucleic acid. During studies on the incorporation of unnatural bases, digests of ribonucleic acid from *Bacterium coli* were found to contain in small amount a compound with spectral and chromatographic characteristics which suggested that it was the riboside of thymine (ref. 4, and Smith, J. D., personal communication). We now have considerable evidence for the natural occurrence of thymine in ribonucleic acid prepared from two strains of *B. coli* and from *Aerobacter aerogenes* and in commercial yeast ribonucleic acid. From these sources we have isolated also three methylated derivatives of adenine: 2-methyladenine, 6-methylaminopurine and 6-dimethylaminopurine. We have found the last two compounds in the ribonucleic acid of rat liver microsomes. None of these compounds appears to be that described previously, which we believe we have noted in addition in ribonucleic acid from several sources. The natural occurrence of all four bases as nucleosides or nucleotides has been described previously, since thymine and 6-methylaminopurine occur in deoxyribonucleic acid[2,5], 2-methyladenine in the vitamin B_{12}-like Factor A[6], and 6-dimethylaminopurine in puromycin[7].

* Scholar in Cancer Research of the American Cancer Society; on leave from the John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Massachusetts.

The ribonucleic acid of *B. coli* has been our main source of these four bases and their derivatives. About ten grams of growing bacteria[8] was collected by centrifugation, washed with saline, and extracted overnight with 60 per cent (v/v) ethanol – water at *p*H 4 and 20°. The residue was washed twice with 60 per cent ethanol – water, and once each with 90 per cent ethanol–water, ethanol, and acetone. After drying, it was extracted with 0·1 *M* sodium citrate for 1–2 hr. at *p*H 7·2 and 100°[9]. The sediment from high-speed centrifugation was discarded and the soluble nucleic acids precipitated three times from 60 per cent ethanol – water at *p*H 4. Two nucleic acid preparations were treated in addition by the Sevag procedure[10] to remove proteins. The final precipitate was hydrolysed overnight at 30° with 1 *N* potassium hydroxide to release the ribonucleoside 2′- and 3′-phosphates. After precipitation of the deoxyribonucleic acid by the addition of two volumes of ethanol plus perchloric and/or acetic acid to *p*H 4, the digest was centrifuged and the soluble nucleotides purified by paper chromatography using *iso*propanol–water (70 : 30, v/v) with ammonia vapour[11].

Early in this work the purified nucleotides were treated with prostatic phosphomonoesterase[12], and the four compounds discovered and studied as nucleosides. In paper chromatography using *iso*-propanol/water with ammonia vapour, *n*-butanol/water (86 : 14, v/v) with ammonia vapour[12], and *n*-butanol/water/formic acid (77 : 13 : 10, v/v/v)[13], each of the four nucleosides showed the same mobility as the corresponding known riboside,

which we synthesized by the incubation of the known base with inosine and a nucleoside phosphorylase prepared from *B. coli*[14]. On paper electrophoresis[11] using 0·05 *M* borate buffer at *p*H 9·2, each nucleoside migrated with the known riboside; in the presence of borate each showed a greatly reduced mobility in *n*-butanol/water/ammonia. The wavelengths of the ultra-violet absorption maxima of these ribosides are given in Table 1. The possibility that we were dealing with deoxyribosides rather than ribosides seems unlikely, since the method of preparation of these compounds involved alkaline hydrolysis of the nucleic acid, and since they formed complexes with borate and moved with the same R_P as the synthetic ribosides in the chromatographic systems mentioned. The deoxyribosides of thymine and 6-methylaminopurine were available and showed greater mobility in the butanol solvents. Furthermore, unlike purine deoxyribosides, the purine ribosides were stable in 0·1 *N* hydrochloric acid at 20°, while they were hydrolysed in 1 *N* hydrochloric acid for 1 hr. at 100° [15]. The nucleoside of thymine was unchanged by this treatment but was hydrolysed in 72 per cent (w/w) aqueous perchloric acid in 2 hr. at 100° [16], or, unlike thymidine, by treatment with sodium periodate followed by alkali[17].

Table 1. Wave-lengths (mμ) of Ultra-violet Absorption Maxima of Methylated Bases and their Ribosides

	Base		Riboside	
	*p*H 1	*p*H 13	*p*H 1	*p*H 13
Thymine	265	291	267	267
2-Methyladenine	266	271	258	262
6-Methylaminopurine	267	273	262	266
6-Dimethylaminopurine	277	281	268	277

The four bases released by hydrolysis of the ribosides had identical ultra-violet absorption spectra (Table 1) and also identical chromatographic behaviour in four solvents to known samples of thymine, 2-methyladenine, 6-methylaminopurine or 6-dimethylaminopurine.

More recently we have isolated what we presume to be the 2′- and 3′-phosphate derivatives of the four ribosides. From the initial *iso*propanol/water/ammonia chromatogram of the soluble nucleotides we eluted the entire area between guanylic acid and the small amount of nucleosides present, and rechromatographed this material for 48 hr. with the same solvent. By this time, two or three faint bands had separated ahead of uridylic acid; these were eluted together and run on paper electrophoresis in 0·05 *M* ammonium formate buffer at *p*H 3·5. In this system the mobilities of the ribonucleotides of thymine, 6-methylaminopurine, 6-dimethylaminopurine and 2-methyladenine were 0·90, 0·46, 0·42 and 0·19 of that of a uridine 2′,3′-phosphate marker. Thus, thymine ribonucleotide was separated from the mixture of the nucleotides of 6-methylaminopurine and 6-dimethylaminopurine, which were then distinguished by chromatography in *iso*propanol/water/ammonia. The ribonucleotide of 2-methyladenine was sometimes present on this electrophoretic separation, but was usually found by similar electrophoresis of the uridylic acid band. In the latter instance, it could be separated from any cytidylic acid present by chromatography in *iso*propanol/water/ammonia. These four ribonucleotides were dephosphorylated by prostatic phosphomonoesterase but not by the 5′-nucleotidase present in rattlesnake venom. The ribosides so produced, as well as the bases released by subsequent acid hydrolysis, were identical to those described above.

Table 2. Molar Proportions of Methylated Bases in Several Ribonucleic Acids

Source of ribonucleic acid	Moles per 100 moles of uracil			
	Thymine	2-Methyladenine	6-Methylaminopurine	6-Dimethylaminopurine
B. coli B/r	0·8	0·5	0·4	0·3
B. coli 15T⁻	0·9	0·25	*	*
Aerobacter aerogenes	1·0	0·25	0·25	0·1
Yeast	0·6	0·15	0·25	0·05
Liver microsomes	—	—	0·65	0·15

* Not examined

In addition to alkaline hydrolysis, we have treated the nucleic acid preparation with rattlesnake venom containing diesterase and 5′-nucleotidase, and have isolated from this digest also the ribosides of thymine (Smith, J. D., personal communication), 2-methyladenine and 6-methylaminopurine, and a small amount of material with the chromatographic behaviour of the riboside of 6-dimethylaminopurine. The preparation of the four ribosides by these two methods strongly suggests that they existed in 3′,5′-phosphodiester linkage, most likely in the polynucleotide chain. This evidence and the thorough washing of the nucleic acid preparation before hydrolysis make it improbable that we are dealing with contaminating free nucleotides. Indeed, it would be unusual for free nucleotides to exist in the 2′- or 3′-phosphate form, and we have searched for and failed to find the ribonucleotide of thymine or 2-methyladenine in the acid ethanol-soluble fraction of the bacteria.

The approximate molar proportion of each base in the five ribonucleic acids we have studied is expressed in Table 2 relative to the amount of uracil. Values were actually determined from the isolated ribosides. Whether the widespread occurrence in ribonucleic acid of small amounts of these four bases is a chance phenomenon or of metabolic importance is not yet apparent.

We are grateful to Dr. D. J. Brown, Dr. D. M. Brown and the American Cyanamid Company for gifts of chemicals, and to Dr. J. D. Smith for much helpful advice. One of us (J. W. L.) wishes to thank the Wellcome Trustees for a travel grant.

[1] Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951). Wyatt, G. R., and Cohen, S. S., *Biochem. J.*, **55**, 774 (1953).
[2] Dunn, D. B., and Smith, J. D., *Nature*, **175**, 336 (1955); *Biochem. J.*, **60**, xvii (1955).
[3] Cohn, W. E., *Fed. Proc.*, **16**, 166 (1957). Davis, F. F., and Allen, F. W., *J. Biol. Chem.*, **227**, 907 (1957).
[4] Dunn, D. B., Ph.D. thesis, University of Cambridge (1957).
[5] Kossel, A., and Neumann, A., *Ber. dtsch. chem. Ges.*, **26**, 2753 (1893).
[6] Dion, H. W., Calkins, D. G., and Pfiffner, J. J., *J. Amer. Chem. Soc.*, **76**, 948 (1954). Brown, F. B., Cain, J. C., Gant, D. E., Parker, L. F. J., and Smith, E. Lester, *Biochem. J.*, **59**, 82 (1955).
[7] Waller, C. W., Fryth, P. W., Hutchings, B. L., and Williams, J. H., *J. Amer. Chem. Soc.*, **75**, 2025 (1953).
[8] Dunn, D. B., and Smith, J. D., *Biochem. J.* (in the press).
[9] Smith, J. D., and Matthews, R. E. F., *Biochem. J.*, **66**, 323 (1957).
[10] Sevag, M. G., Lackman, D. B., and Smolens, J., *J. Biol. Chem.*, **124**, 425 (1938).
[11] Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 552 (1952).
[12] Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 558 (1952).
[13] Markham, R., and Smith, J. D., *Biochem. J.*, **45**, 294 (1949).
[14] Paege, L. M., and Schlenk, F., *Arch. Biochem. Biophys.*, **40**, 42 (1952).
[15] Vischer, E., and Chargaff, E., *J. Biol. Chem.*, **176**, 715 (1948).
[16] Marshak, A., and Vogel, H. J., *J. Biol. Chem.*, **189**, 597 (1951).
[17] Whitfeld, P. R., *Biochem. J.*, **58**, 390 (1954).

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Iodine-131 in Sheep before and after a Nuclear Reactor Accident

It may be useful to compare the amount of iodine-131 in thyroids of unselected sheep slaughtered in London before and after the release of iodine from the reactor at Windscale in Cumberland on October 10, 1957 [1].

Table 1. Iodine-131 in Sheep Thyroid Glands (mμc./gm. Thyroid)

London				Munich				Nashville			
Date slaughtered (day/month)	No. of sheep	Iodine-131 in thyroids		Date slaughtered (day/month)	No. of sheep	Iodine-131 in thyroids		Date slaughtered (day/month)	No. of sheep	Iodine-131 in thyroids	
		Average	Max./min.			Average	Max./min.			Average	Max./min.
14/8	8	0·99	1·9/0·54					12/8	14	28	145/13
6/9	8	1·0	2·3/0·76					6/9	11	12	24/5·3
10/10	Windscale release							9/10	17	55*	84/19
21/10	15	15	59/5·9					21/10	13	14	48/7·5
				7/11	6	0·36	0·70/0·07	4/11	9	7·2	26/1·4
23/11	11	2·3	6·8/0·74	26/11	4	0·24	0·58/0·20	25/11	14	0·83	2·6/0·21
				8/12	6	0·10	0·2/0·05	10/12	11	0·23	0·44/0·10
20/12	26	0·14	0·19/0·12					20/12	14	0·09	0·19/0·07

* Maximum for the period September–December.

For three years our laboratory has frequently measured the iodine-131 in thyroids of sheep and cattle slaughtered in certain cities of the United States, London, Japan and Germany (Munich).

During the summer of 1957, the sheep thyroids received once each month from London contained 1–10 per cent of the amount present in thyroids from Nashville, Tennessee. This radioactivity was considered to originate west of Tennessee. Similar results were published in 1956 [2]. The reduced amount in the English and Munich animals was possibly due to the three to four additional half-lives of time necessary for the isotope to appear in the sheep of the more distant areas.

Table 1 shows that in August and September of 1957 the concentrations of iodine-131 in thyroids of sheep slaughtered in London averaged 1 mμc./gm. This was less than one-tenth of the concentrations found during the same period in specimens from Tennessee. In October the greatest concentrations in thyroids of Tennessee sheep averaged 55 mμc./gm., on October 9, the day before the Windscale incident. Most of this iodine-131 is believed to have originated from nuclear weapons tests, 1,500 miles west of Tennessee. Ten days after the Windscale release, our specimens of British and American sheep thyroids both averaged 14–15 mμc./gm.

Sheep slaughtered in Munich in early November contained 1–10 per cent as much iodine-131 as the average of Tennessee sheep. This suggests that most of the iodine-131 in the German animals could have originated west of Tennessee. The concentrations of iodine-131 in German sheep have decayed more slowly than those in Tennessee sheep. This slow decay is interesting but not unexplained.

In general, these results suggest that sheep slaughtered in London have received less iodine-131 from the Windscale incident than sheep slaughtered in Tennessee have received from distant nuclear weapons tests.

In the United States, animals grazing east of the Nevada proving ground are more sensitive indicators to iodine-131 fall-out than those west of the test site. Therefore, directly east of Windscale, sheep may have contained more iodine-131 than those reported here. It is further suggested that valuable information can be obtained by continuous monitoring of thyroids from slaughtered sheep and cattle. A good index is thus obtained regarding the biological concentration of air-borne radioactive fall-out.

This investigation was supported by grants from the U.S. Atomic Energy Commission and the U.S. Public Health Service.

Thyroid glands were sent to us by Mr. E. F. McCleary, of the Metropolitan Cattle Market, London; Herr Schurry, of Max Muhlpointner and Co., Munich, Germany; and the Neuhoff Packing Company (Division of Swift and Co.), Nashville, Tennessee.

L. Van Middlesworth

Department of Physiology,
University of Tennessee,
Memphis, Tennessee.

[1] Accident at Windscale No. 1 Pile on 10th October 1957. Cmnd. 302. (H.M. Stationery Office, London, 1957.)

[2] Van Middlesworth, L., *Science*, **123**, 982 (1956).

Symmetry of Snow Crystals

The frequently portrayed beautiful six-ray star-shaped crystals of snow have long been quoted as outstanding examples of well-developed crystal forms. Despite an infinite variety in the patterns which appear, there does often exist a remarkable symmetry in the six rays, the complex tree-like pattern repeating closely in each arm, indeed, surprisingly so. Since each of the six arms of the crystal would appear to be growing independently, this symmetry poses a problem in crystal growth, for it almost seems as if each arm of the six-ray star 'knows' what the other five are doing and follows suit. In fact I believe that each arm does 'know' what the others are doing and I offer the following explanation.

I conjecture that the crystal is vibrating mechanically as a flat plate, in fact as a Chladni plate, with of course a hexagonal symmetry. Such a mechanically vibrating system will have a complex, yet fundamentally hexagonal, distribution of nodes and anti-nodes. A growing crystal is in a dynamic state, being bombarded by water-vapour molecules, some of which can and do adhere locally to promote local growth. When the molecules adhere to achieve growth at any particular region on one arm, this immediately introduces a localized damping action on the vibrations. One can certainly anticipate that in a delicate equilibrium water molecules are less likely to adhere in regions in violent motion, and

more likely to adhere in nodal or damped regions. Thus once growth starts it has a tendency to continue.

But this very damping is at once felt simultaneously at the corresponding positions on the other five arms. Thus molecules arrive and adhere easily at the other five arms in precisely the same situations as on the first arm; in other words, what happens on any one arm tends to be repeated on the others; hence the symmetrical growth, despite the apparent independence of each arm. The final symmetry is then explicable purely in terms of localized damping of mechanical vibrations, provided of course that the crystal is growing in conditions of delicate equilibrium, which is doubtless the case with the very perfect examples often found.

Of course, it is possible to produce alternative explanations; but they appear far-fetched. For example, to assume that some identical dislocation factor appears throughout the whole complex pattern and therefore simultaneously influences each arm seems most unreasonable and untenable. To postulate that ambient variations (temperature concentration, etc.) could identically affect each arm at every point simultaneously seems also unrealistic.

If the conjecture as to mechanical vibration, offered here, is correct, there would appear to be several implications. Thus: (1) The freely falling snowflake, being unconstrained to vibrate, might be expected in general to be more symmetrical than one grown on a solid surface. (2) The mechanical vibration of the crystal might conceivably be detectable as an ultra-sonic sound vibration. The dimensions of typical snowflakes indicate that these would be of very high frequency indeed, would have a complex spectrum, and doubtless would have very small amplitude. Indeed the detection of any such radiation would offer a most formidable problem. (3) On the other hand, if growing vibrating snowflakes are subjected to the right kind of ultra-sonic frequency, it might well be expected that the growth symmetry would be markedly affected.

S. Tolansky

Royal Holloway College,
Egham, Surrey.
Dec. 23.

A Photographic Observation of the Satellite 1957 Beta leaving the Earth's Shadow

On December 15 a photograph of the satellite 1957 Beta was taken at the Warner and Swasey Observatory of the Case Institute of Technology, Cleveland, Ohio, which shows the satellite coming out of the Earth's shadow (Fig. 1). This observation can be used for deriving the distance of the satellite from the Earth's surface. It was concluded from visual observations made the previous morning that the satellite should leave the Earth's shadow in the constellation Ursa Major. An exposure, therefore, was started at 5h. 10m. 00s. E.S.T. with Ursa Major in the centre of the field, although the satellite was not yet visible. The satellite was first observed visually between 5h. 10m. 20s. and 5h. 10m. 30s. It is estimated that it came out of the shadow not earlier than 5h. 10m. 10s. The position where the track is first recorded on the photograph is $\alpha = 11\text{h. } 53\text{m.}$, $\delta = +54{\cdot}8°$. It is possible that the satellite at this position had already left the shadow, but was not recorded earlier because it was at minimum brightness. Nevertheless, since the period of light variation was about 40 sec., the real position should be between the value already given and $\alpha = 11\text{h. } 37\text{m.}$, $\delta = +61°$. From these two positions, heights above the surface of 770 and 800 km. respectively were derived, assuming a spherical Earth and a cylindrical shadow with a radius equal to that of the Earth. If refraction is taken into account, smaller values of the height result. Assuming a deflexion of the light rays from the Sun of 1°, the corresponding heights are 713 and 739 km. It would appear then that the derived value of 785 ± 15 km. is a maximum one. From predictions of the Smithsonian Institution a height of about 1,000 km. was expected. According to our observations this is too large by at least 200 km.

Fig. 1. Photograph of the satellite 1957 Beta coming out of the Earth's shadow near γ Ursae Majoris on December 15. Exposure time: 5h. 10m. 00s. to 5h. 10m. 58s. E.S.T. (35-mm. camera, $f/2{\cdot}8$; Kodak 'Tri-X' film)

From observations on December 14 and 15 a period of 101·7 min. and, therefore, a value of 7,310 km. for the semi-major axis have been derived. Assuming the distance at perigee of 6,543 km. computed from the elements given in Harvard Announcement Card 1384 has not changed, the computed value of the eccentricity is $e = 0{\cdot}0927$. With these values the true anomaly can be derived for a given height. With the value of the inclination (62·5°) also given in Harvard Announcement Card 1384, the argument of perigee can be derived. While a height of 1,000 km. gives for the true anomaly and argument of perigee 109° and 22·5°, respectively, the corresponding values of 90·5° and 41° follow from these observations. The argument of perigee at November 4·0 was 44° according to Harvard Announcement Card 1384. It follows that the value of regression of the perigee is only $-0{\cdot}07°$/day according to our observations, whereas a height of 1,000 km. gives a rate of $-0{\cdot}52°$/day, close to the value of $-0{\cdot}6°$/day given in Harvard Announcement Card 1384.

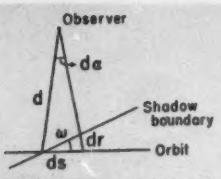


Fig. 2. The error in height as computed from the error in position. $ds \approx (2\pi d/360)\ d\alpha$; $dr \approx \tan\omega\ ds$. With $\omega_{max.} = 27{\cdot}3°$ and $d = 800$ km., $dr/d\alpha = 7{\cdot}2$ km./deg.

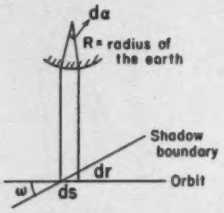


Fig. 3. The error in height as computed from the error in time. In the time dt (in min.) a point on the Earth's surface at latitude φ has moved by the amount $ds = 2\pi/360\ R\cos\varphi\ d\alpha = 2\pi/360\ R\cos\varphi\ dt/4$. $dr = \tan\omega\ ds$. $dr/dt = 10{\cdot}3$ km./min. for the latitude of Cleveland

Observations of a shadow-egress give very accurate values for the height of a satellite above the Earth's surface. At a height of 800 km. the largest angle possible between the orbit and the boundary of the shadow is 27·3°. From Figs. 2 and 3 it follows that an error in the observed position of egress of one degree results in a maximum error of 7·2 km. in the distance. An error of one minute in time results in an error of 10·8 km. in distance. Therefore, even approximate observations give rather accurate values for the height of a satellite, at least within ± 30 km. The height of 1,000 km. which was expected would require that the satellite had already left the shadow at a distance of 30° from our first observed position. There seems to be no reason why the satellite should not have been visible if this were correct.

The method used for the derivation is shown in Fig. 4. From the observed position of egress, the sidereal time, the latitude of the observer, and the spherical co-ordinates of the Sun, rectangular co-ordinates can be derived for the points F and H. The equations for the line $\overline{OH}$ give the direction toward the observed position of egress. The intersection of a line through F parallel to $\overline{OH}$ with the cylindrical or conical shadow gives the rectangular co-ordinates X_s, Y_s, Z_s for the satellite leaving the shadow. The distance from the Earth's centre is then $r = (X_s^2 + Y_s^2 + Z_s^2)^{1/2}$.

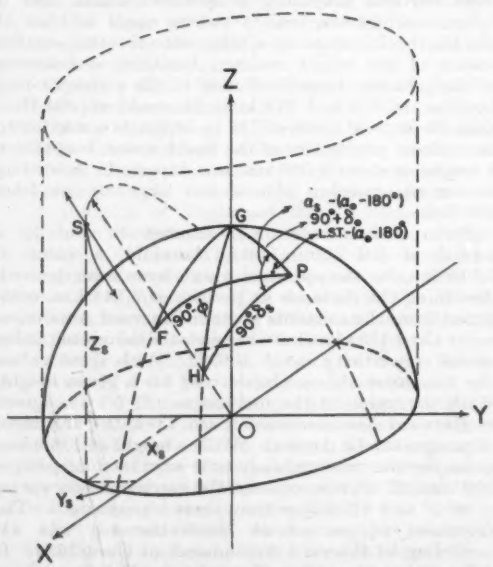


Fig. 4. Derivation of the height of a satellite above the Earth's surface. F, observer; P, pole of the Earth; G, point opposite to the Sun; H, point with the observed egress position in the zenith; S, satellite leaving the shadow; φ, observer's latitude; a_s, δ_s, right ascension and declination of the observed position of S; $a\odot$, $\delta\odot$, right ascension and declination of the Sun; L.S.T., local sidereal time

Heinz Neckel

Warner and Swasey Observatory,
East Cleveland 12,
Ohio. Dec. 21.

Precision Determination of the Velocity of Electromagnetic Waves

The free-space phase velocity of electromagnetic waves *in vacuo* has been measured by means of a millimetre-wave interferometer operating at a frequency of 72 Gc./s., corresponding to a wave-length of about 4 mm. The result is:

$$c_0 = 299\,792{\cdot}50 \pm 0{\cdot}10 \text{ km./s.}$$

The variation represents the standard deviation of a single observation in statistical combination with estimated systematic uncertainties, the greatest of these being in the measurement of the standards of length used (derived by optical interferometry), and in the measurement of the refractive index of the air surrounding the microwave interferometer (obtained by means of a microwave refractometer).

The 24-Gc./s. prototype instrument has already been described[1], the 72-Gc./s. interferometer being basically identical, but considerably improved in performance. A full report of the present determination will be published in due course, but it should be noted here that the value previously assigned to the one-metre length-standard used with the prototype interferometer was too great, and this has caused the published value of c_0 obtained from that instrument to be high by 0·2 km./s. Thus the corrected value of the earlier determination[1] is: $c_0 = 299\,792{\cdot}7_5 \pm 0{\cdot}3$ km./s.

This work has been carried out as part of the research programme of the National Physical Laboratory and is published by permission of the Director of the Laboratory.

K. D. Froome

Metrology Division,
National Physical Laboratory,
Teddington, Middlesex.
Nov. 5.

[1] Froome, K. D., *Proc. Roy. Soc.*, A, 223, 195 (1954).

Metallographic Studies of Iron Carbide and Tungsten Carbide

In most metallographic investigations of steels or of cemented carbides the fine details of the carbides are not studied. We have just completed an etching study of iron carbides made in three different ways and of some tungsten carbide (1/32-in. equilateral triangles) single crystals. The iron carbide studies, made with Carapella's reagent (5 gm. ferric chloride,

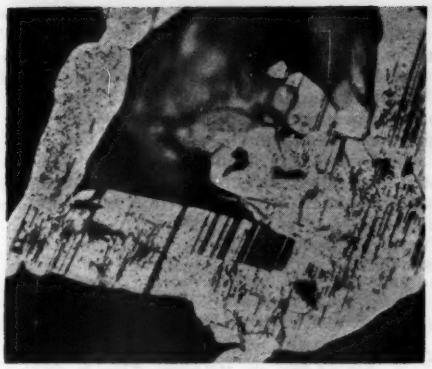


Fig. 1. Striæ in carburized iron ('Puron') carbide. Carapella's reagent. (× 1,000)

2 ml. concentrated hydrochloric acid and 99 ml. methyl alcohol) etching for 20–60 min., revealed cracking as the principal deformation mechanism and twinning as the secondary agency. The cracking behaviour has been described previously, but the twinning appears to be a new observation[1]. In two cases only, out of hundreds of samples studied, the cracks found were aligned along the twinning planes in the carbides. These observations were made upon a carburized plain carbon steel, upon a carburized 'Westinghouse Puron' iron (spectrographically pure) and upon a pure, massive, polycrystalline, iron-manganese carbide kindly supplied by Dr. L. S. Darken of U.S. Steel Research from his recent ternary system studies. In the two carburized samples there was no possibility of mechanical work having caused cracking of the carbide. Fused caustic, 50 per cent nitric acid and 50 per cent hydrochloric acid were also successful etching reagents.

The tungsten carbide crystals etched nicely in fused sodium hydroxide held just above its melting point in a nickel crucible heated electrically by a knife-blade type heater insulated with glass wool and controlled by a 'Variac'. This reagent and technique also etched pure tantalum carbide crystals. Only the fused caustic served as an etching reagent for the tungsten carbide crystals. After this caustic etching, the sample surfaces were cleaned in an acid solution, either 'Nital' or Carapella's reagent being satisfactory. For those who are concerned with hard carbides, cemented carbides, or the production of carbide die patterns, this fused caustic etching technique may prove useful.

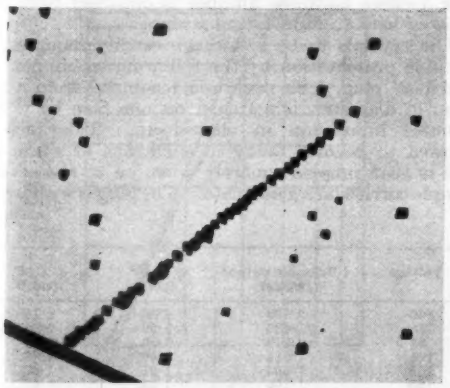


Fig. 2. Etch-pit array (dislocation line?) in iron–manganese carbide. Dark solid line in lower left is a grain boundary. Carapella's reagent. (× 625)

We believe that the twins in the iron carbides are an index of the carbide orientation and morphology. Most of the large iron carbides present in the steels are single crystals of irregular shape. The cracking and the presence of twins indicate high internal stresses.

F. J. Radd
L. H. Crowder

Central Research Division,
Development and Research Department,
Continental Oil Company,
Ponca City, Oklahoma.
Oct. 21.

[1] Hopkins, B. E., *West of Scotland Iron and Steel Inst. J.*, discussion, **60**, 340 (1953).

Two Borides with the Cementite Structure

An investigation by X-ray powder methods of the nickel–boron and the cobalt–boron systems has revealed the existence of two isomorphous borides, Ni_3B and Co_3B. A nickel boride with a boron content about 25 atom per cent has previously been reported by Andersson and Kiessling[1], but no reports on Co_3B seem to have been published.

Alloys were prepared by heating mixtures of amorphous boron (99·1 per cent) with nickel powder (99·8 per cent) or cobalt powder (99·2 per cent, containing 0·45 per cent nickel) in an argon-arc furnace or in evacuated and sealed silica tubes. X-ray diffraction patterns were recorded in chromium K_α and copper K_α radiation with Guinier-type focusing cameras.

The orthorhombic unit cell of Ni_3B has the dimensions $a = 4·389$ A.; $b = 5·211$ A.; $c = 6·619$ A. The lattice dimensions of this phase showed no significant variations in alloys of different composition. The unit cell of Co_3B was found to vary between $a = 4·408$ A.; $b = 5·223$ A.; $c = 6·629$ A. and $a = 4·411$ A.; $b = 5·235$ A.; $c = 6·634$ A. The lattice parameter values are probably accurate to 0·05 per cent. As seen from Table 1, the visually estimated intensities of the lines are in qualitative agreement with the powder data for cementite (Fe_3C) given by Lipson and Petch[2].

It is interesting to note that up to 80 per cent of the carbon in Fe_3C can be substituted by boron while retaining the cementite structure[3]. No binary boride Fe_3B seems to exist, but it may be mentioned that in the ternary system iron/boron/phosphorus a phase

Table 1. Intensity Observation for Co_3B (Chromium $K\alpha$ Radiation) compared with Data for Fe_3C given by Lipson and Petch (Ref. 2)

hkl	$I_{obs.}$ Co_3B	$I_{obs.}$ Fe_3C	*hkl*	$I_{obs.}$ Co_3B	$I_{obs.}$ Fe_3C
101	*v.w.*	—	103	*st.*+	23·2
002	*w.*	—	211	*st.*	12·6
111	*w.*	—	122	*st.*	9·2
020	*w.*	1·0	113	*st.*	7·2
021	*m.*	} 14·4	202	*w.*	—
112	*st.*		212	*st.*	3·8
120	*m.*	5·8	023	*m.*	} 3·8
200	*w.*	6·3	004	*m.*	
121	*st.*	14·2	221	*v.w.*	1·6
022	*st.*	14·2	130	*m.*	4·5
210	*st.*	15·6			

v.w., very weak; *w.*, weak; *m.*, moderate; *st.*, strong.

$Fe_xB_2P_{1-x}$ with variable composition ($0{\cdot}5 < x < 1$) has been found which exists with phosphorus content less than 1 atom per cent (unpublished work). The structure of this phase, based on a primitive tetragonal lattice, shows no obvious resemblance to the cementite structure, however.

A detailed report of this work, together with a structure determination of Ni_2B from single-crystal data, will soon be published in *Acta Chemica Scandinavica*.

This work has been supported by a scholarship from Statens Tekniska Forskningsråd, which is gratefully acknowledged.

Stig Rundqvist

Inorganic Department,
Institute of Chemistry,
University of Uppsala,
Uppsala.
Oct. 8.

[1] Andersson, L.-H., and Kiessling, R., *Acta Chem. Scand.*, **4**, 160 (1950).
[2] Lipson, H., and Petch, N. J., *J. Iron and Steel Inst.*, **142** (II), 95 (1940).
[3] Nicholson, M. E., *J. Metals*, **9** (Jan. 1957).

Amplitude of a Quartz Plate vibrating in Liquids

Some attempts have been made to measure the amplitude of a vibrating quartz crystal, but no method at present exists by which its absolute value in a liquid can be determined experimentally, although the amplitude can be calculated theoretically from the relation given by Vigoureux[1]:

$$a = \frac{2c_{11}Vd}{\pi c_1 \rho_c}$$

where c_{11} is the elastic modulus governing the thickness vibration of X-cut plate; d the change in thickness of the plate per volt ($2\mu\mu$m.); V the voltage applied; c_1 the velocity of sound in quartz and ρ_c the radiation resistance of the transmission medium.

The development of a thermosonic method in this laboratory has now enabled us to determine the amplitude of vibration. The principle of the method lies in evaluating the amplitude of the plane sound-wave at the source of the ultrasonic beam from the mean power of the sound from the formula[2]

$$P = 2\pi^2 \rho_c \xi^2 N^2 S$$

where N is the frequency; ξ the amplitude and S the area of source of sound. The mean power of the sound, P, is obtained from the relation[3]: $I = JH$, which involves the measurement of heat produced by a calorimetric method.

The amplitude of a quartz plate as determined from Vigoureux's formula and the amplitude of sound calculated from power measurements of the output of sound, carried out in this laboratory[4] for 1 V. input at about 5 Mc. are given in Table 1.

These results show that the agreement between the amplitudes of oscillation of the quartz and of sound is good. It is observed that the amplitude of the sound wave is always less than that of the quartz, the variation lying between 10 and 25 per cent. It is also noticed that the difference between the two amplitudes, albeit small, varies with the nature of the liquid used. It is likely that this variation is related to some property of the liquid or of the quartz crystal, although it may be due to experimental limitations.

Further investigations are in progress, and full details will be published elsewhere.

S. Parthasarathy
Harkrishan Singh

National Physical Laboratory
of India,
New Delhi.
Sept. 30.

[1] Vigoureux, P., "Ultrasonics" (1950).
[2] Wood, A. B., "A Textbook of Sound" (1955).
[3] Parthasarathy, *et al.*, *Nature*, **166**, 829 (1950); *Ann. Phys.*, **12**, 8 (1953); *Z. Phys.*, **135**, 395 (1953).
[4] Parthasarathy, S., and Narasimhan, V., *Z. Phys.*, **143**, 300 (1955).

Table 1

Liquid	c (m./sec.)	ρ (gm./sec.^{3})	$\rho_c \times 10^{-5}$	Voltage	Ultrasonic output (watts)	$\xi \times 10^5$ (cm.)	$a \times 10^5$ (cm.)
Methyl alcohol	1,123	0·792	88·9	160	2·393	1·96	2·18
Acetone	1,192	0·792	94	158	1·626	1·59	2·06
Methyl ethyl ketone	1,207	0·805	97·2	94·5	0·579	1·56	1·99
Butyl alcohol (*n*)	1,268	0·810	103	165	1·514	1·40	1·88
Xylene (*m*)	1,340	0·863	116	158	1·364	1·31	1·67
Benzene	1,326	0·878	116	155	1·199	1·25	1·67
Chlorobenzene	1,291	1·107	143	162	1·308	1·13	1·36
Carbon tetrachloride	938	1·595	150	173	1·361	1·05	1·29
Glycerine	1,923	1·261	242	173	1·026	0·72	0·80

Surface Hardness of Explosive Materials by Micro-indentation

The influence of adulterating grit on the impact and friction sensitivity of a number of explosives has been shown to be considerable[1]. It is generally agreed that grit sensitizers act through modification of conditions necessary to produce frictional hot-spots, and that this process is influenced to a large extent by the hardness of the grits. Although detailed hardness data are now available for many of the common grits[2], little or none exists for the explosives themselves.

The following technique, designed to follow the modifying effect of X-rays and light on the hardness of α-lead azide, makes such detailed information readily available in many instances. The apparatus used is the Cooke, Troughton and Simms micro-indentation hardness tester fitted to a Vickers projection microscope[3]. With this instrument, materials may be indented at known load with a diamond pyramidal indenter and the indentation measured. The Vickers hardness number is obtained from the ratio of load to indentation surface.

The crystals to be tested are readily mounted in suitable presentation by the following technique. A 'Perspex' plug, $\frac{1}{4}$ in. deep and machined from a rod 1 in. in diameter, is softened on one face by a few minutes immersion in chloroform. This face is allowed to become tacky in a current of warm air and is then pressed squarely down on to the crystal sample carried on a glass plate. The plug is withdrawn

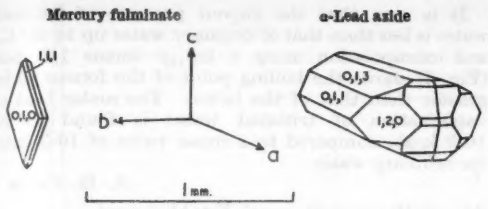


Fig. 1

and the crystals allowed to set hard. In this way, principal faces are oriented away from the plug and suitable for indentation. Lesser faces may be brought into orientation while the 'Perspex' is still plastic by careful probing under a microscope.

The plug is now inserted in the chuck of the micro-indenter and indented in standard fashion.

Small fragments of annealed 'from the melt' crystals of sodium chloride and lithium fluoride were selected as controls and mounted and indented by the above method. These two salts have hardness values of an order similar to those of the initiators mercury fulminate and α-lead azide. Vickers hardness numbers of the 1,0,0 forms of sodium chloride and lithium fluoride for an indentation diagonal of 30μ were found to be 19·5 and 103 respectively in good agreement with published values[4]. No surface hardening was observed, the apparent Meyer coefficients being widely constant over the loading range at 2·04 and 2·13 respectively.

Crystals of the two important initiators, mercury fulminate and α-lead azide, were selected for examination. Crystals of the pure material, larger than normal, were grown by crystallization from solution in aqueous ammonium chloride and ammonium acetate respectively. The faces examined were those principally defined in material used for Service purposes, and were distributed as shown in Fig. 1.

A number of crystals of each initiator were mounted and indented at various loads, results from several crystals and by two operators being incorporated in the results. The result for a 10-gm. load on the 0,1,3

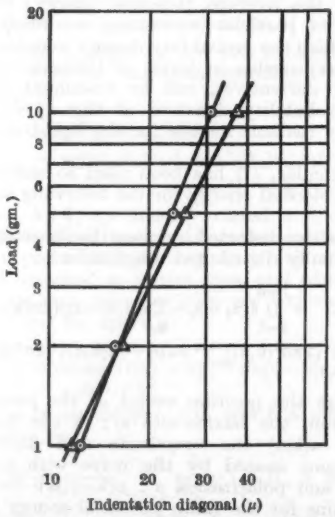


Fig. 2. A log/log plot of indentation diagonal against appropriate load for mercury fulminate: ○, for 1,1,1 form; △, for 0,1,0 form

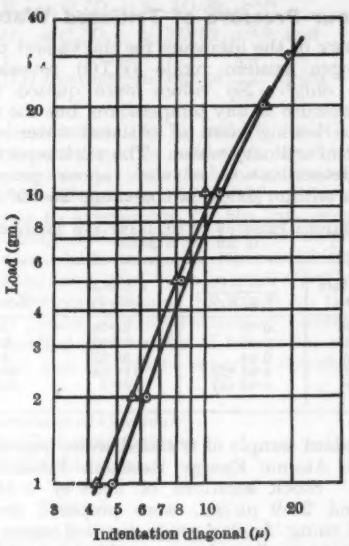


Fig. 3. A log/log plot of indentation diagonal against appropriate load for α-lead azide. ○, for 0,1,1/0,1,3 forms (individual curves irresolvable); △, for 1,2,0 form

form of α-lead azide is a particularly well-established value, being the result of seventy-five determinations. Figs. 2 and 3 show the plot of the logarithms of load against indentation diameter for the appropriate crystal forms.

Where the gradient of this line is uniform and equal to 2, the Vickers hardness number is constant for all loads: otherwise, it is variable and must be quoted against experimental conditions. In the present context, it is convenient to present the hardness figures against standard load and this has been done in Table 1.

Table 1

Initiator	Miller indices of crystal form	Vickers hardness number			
		At 1-gm. load	At 5-gm. load	At 10-gm. load	At 20-gm. load
Mercury fulminate	0,1,0	13·5	14·1	14·4	—
	1,1,1	12·3	17·4	19·9	—
α-Lead azide	0,1,1 } 0,1,3 }	80	131	144	145
	1,2,0,	99	151	165	161

These hardness values relate to material substantially unexposed to light. We have shown (unpublished work) that the hardness of α-lead azide is severely modified by exposure to soft X-rays, and related questions such as the effect of exposure to ultra-violet light are being investigated.

G. Todd
E. Parry

Ministry of Supply,
Armament Research and Development Establishment,
Fort Halstead. Nov. 4.

[1] Taylor, W., and Weale, A., *Trans. Farad. Soc.*, **34**, 995 (1938). Copp, J. L., and Ubbelohde, A. R., *Phil. Trans.*, A, **241**, 248 (1948). Bowden, F. P., and Gurton, O. A., *Nature*, **162**, 654 (1948).
[2] Mott, B. W., "Micro-indentation Hardness Testing", Appendix (Butterworth, 1956).
[3] Mott, B. W., "Micro-Indentation Hardness Testing", p. 23 (Butterworth, 1956).
[4] Leise, K. H., *Z. Phys.*, **124**, 258 (1946).

Vapour Pressure of Tritiated Water

A survey of the literature for the vapour pressure of hydrogen tritium oxide (HTO) revealed one reference only[1]. No values were quoted for the vapour pressure at any temperature, but the authors stated the boiling point of tritiated water was less than that of ordinary water. The work reported here is the determination of the vapour pressure of hydrogen tritium oxide in the range 25–80° C.

Table 1. Vapour Pressure of Ordinary and Tritiated Water in mm. of Mercury

Temperature (° C.)	V.p. HTO / V.p. H_2O	V.p. H_2O (ref. 3)	V.p. HTO
25·70	0·77	24·76	19·07
27·84	0·81	28·05	22·72
39·98	0·84	55·27	46·43
55·02	0·88 (5)	118·04	104·47
75·60	0·89 (5)	296·3	265·2

A standard sample of tritiated water was obtained from the Atomic Energy Research Establishment, Harwell. Stock solutions of activity 4·84, 9·61, 16·75 and 24·9 μc./ml. were prepared from this standard using 'Analar'-grade distilled water. These samples were allowed to equilibriate with their vapours in a glass apparatus totally enclosed in a thermostated bath. Using this apparatus, it was not found possible to make measurements above 80° C. due to 'streaking' occurring in the high-vacuum grease used to lubricate the glass stopcocks and joints. The water vapour was isolated from the liquid phase and analysed by the butane method of Glascock[2]. At any one temperature the ratio of the activity in the vapour to that in the liquid was constant, showing that the system obeyed Henry's law. It may be shown that this ratio equals the ratio of the saturation vapour pressure of tritiated to that of ordinary water. The results are given in Table 1.

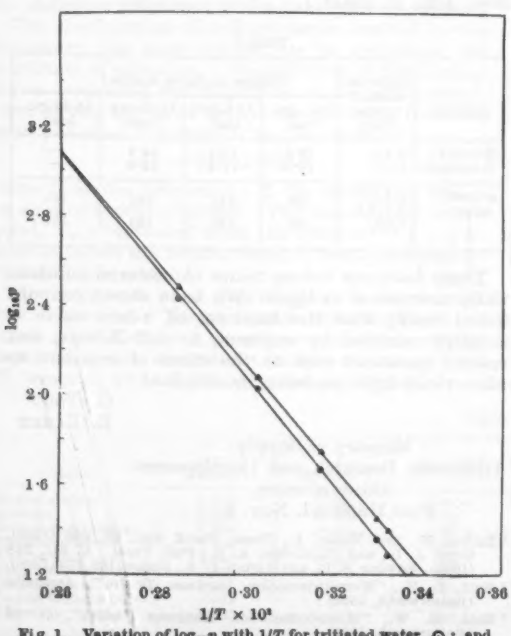


Fig. 1. Variation of $\log_{10}p$ with $1/T$ for tritiated water, ⊙; and for ordinary water, △

It is seen that the vapour pressure of tritiated water is less than that of ordinary water up to 80° C., and extrapolation using a $\log_{10}p$ versus $1/T$ plot (Fig. 1) shows the boiling point of the former to be greater than that of the latter. The molar heat of vaporization of tritiated water is found to be 10·9 kcal., compared to a mean value of 10·3 kcal. for ordinary water.

A. H. Price

Atomic Weapons Research Establishment,
Aldermaston,
Berks.
Nov. 8.

[1] Libby, W. F., and Cornog, R., *Phys. Rev.*, **60**, 171 (1941).
[2] Glascock, R. F., "Isotopic Gas Analysis", Chapter 8 (Academic Press, Inc., New York).
[3] Int. Crit. Tables, **3**, p. 212.

Use of Gaussian Functions for Intermolecular Potentials

The purpose of this communication is to point out that the superposition of two Gaussian functions,

$$\varphi(r)^G = -C_1 \exp(-c_1 r^2) + C_2 \exp(-c_2 r^2) \quad (1)$$

with suitably chosen constants (C_1, c_1, C_2, $c_2 > 0$) provides a good approximation to the Lennard-Jones potential,

$$\varphi(r)^{L\text{-}J} = -a\,r^{-6} + b\,r^{-12} \quad (2)$$

which is approximately valid for the interaction between spherically symmetrical molecules; and that the use of (1) leads to comparatively simple expressions in certain types of theoretical work.

Fig. 1 shows that the agreement between (1) and (2) is close except at large values of r where $|\varphi(r)|$ has become small; the fact that (1) remains finite as $r \to 0$ does not prevent a sufficiently rapid increase with decreasing r in the practically important range. The question whether the larger number of adjustable constants in (1) offers any advantage in the direct correlation of experimental data has not been investigated.

A mathematical advantage of (1) arises from the fact that the average potential energy, $\overline{\Phi}$, of an assembly of particles interacting according to (1), and for which the probability density in configuration space is expressible in terms of Gaussian functions and their derivatives, can be evaluated in closed form. Probability densities of this type may be reasonably realistic models for the liquid (and solid) state.

In particular, (1) has been used to calculate the average potential energy for the following statistical models:

(*a*) A lattice distorted by plane displacement waves with normally distributed amplitudes:

$$\rho(..\mathbf{x}_j,..) = \prod_{j=1}^{j=n} \delta[\mathbf{x}_j - \mathbf{s}_j - \sum_{\mathbf{k},\alpha} \mathbf{a}(\mathbf{k},\alpha) \exp(2\pi i \mathbf{k}.\mathbf{s}_j)] \prod_{\mathbf{k},\alpha} [2\pi\sigma^2(\mathbf{k},\alpha)]^{-1/2} \exp\{-|\mathbf{a}(\mathbf{k},\alpha)|^2/2\sigma^2(\mathbf{k},\alpha)\} \quad (3)$$

($\mathbf{x}_j$ denotes the position vector of the particle displaced from the lattice site $\mathbf{s}_j$; δ the Kronecker symbol; $\mathbf{a}(\mathbf{k},\alpha)$ the amplitude and direction of displacement caused by the wave with the wave vector $\mathbf{k}$ and polarization α; $\sigma(\mathbf{k},\alpha)$ are constants). One obtains for the mean potential energy between two particles attached to the lattice sites $\mathbf{s}_j$ and $\mathbf{s}_{j'}$ an expression of the form:

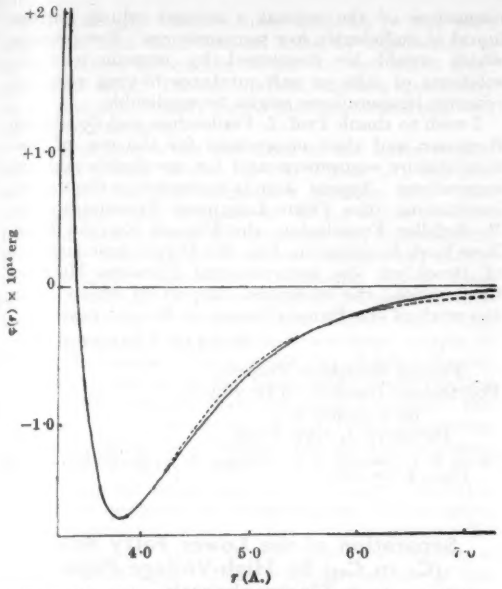


Fig. 1. Comparison of $\varphi(r)^{\mathcal{G}}$ and $\varphi(r)^{L\text{-}J}$ for argon: – – –, $\varphi^{L\text{-}J} = -1{\cdot}05 \times 10^{-10}\, r^{-6} + 1{\cdot}63 \times 10^{-7}\, r^{-12}$ (see ref. 1); ———, $\varphi^{\mathcal{G}} = -9{\cdot}12 \times 10^{-14} \times \exp(-0{\cdot}106\, r^2) + 2{\cdot}40 \times 10^{-10} \times \exp(-0{\cdot}783\, r^2)$. φ in ergs in each case, r in Ångström units

$$\bar{\Phi}_{jj'} = -C_1\, g(c_1)^{-3/2} \times \exp\{-|s_j - s_{j'}|^2\, f(c_1)/g(c_1)\} + C_2\, g(c_2)^{-3/2} \times \exp\{-|s_j - s_{j'}|^2\, f(c_2)/g(c_2)\} \quad (4)$$

where f and g are polynomials of second and third degree respectively in the $\sigma^2(\mathbf{k},\alpha)$'s and in c_1 or c_2. The coefficients in these polynomials are determined by the lattice-type and by the lattice vector $(s_j - s_{j'})$.

(*b*) A lattice in which the displacements of particles are mutually correlated and normally distributed:

$$\rho(.,\mathbf{x}_j,.) = (2\pi)^{-3n/2}\|Q_{ij}\|^{-1/2} \exp\{-1/2 \sum_j \sum_{j'} \sum_\alpha \sum_{\alpha'} (x_j^\alpha - s_j^\alpha) Q_{jj'}^{\alpha\alpha'} (x_{j'}^{\alpha'} - s_{j'}^{\alpha'})\} \quad (5)$$

where x_j^α, s_j^α are the α-components of the vectors $\mathbf{x}_j$, $\mathbf{s}_j$; $Q_{jj'}^{\alpha\alpha'}$ are (constant) elements of a symmetrical matrix which depends on the symmetry of the lattice; $\|Q\|$ is the [corresponding determinant. This leads to

$$\bar{\Phi}_{jj'} = -C_1\{h_1(c_1).h_2(c_1).h_3(c_1)\}^{-1/2} \exp\{-|s_j - s_{j'}|^2.c_1/h_1(c_1)\} + C_2\{h_1(c_2).h_2(c_2).h_3(c_2)\}^{-1/2} \exp\{-|s_j - s_{j'}|^2 c_2/h_1(c_2)\} \quad (6)$$

where h_1, h_2, h_3 are linearly related to c_1 or c_2 and to the reciprocals of linear combinations of the $Q_{jj'}^{\alpha\alpha'}$.

(*c*) A probability density in which the particles are not attached to lattice sites, and which tends to zero when any two particles are too close to each other or too far apart:

$$\rho(.,\mathbf{x}_j,.) = \text{const.} \prod_{j=1,\, j'>j}^{j=n,\, j'=n} |\mathbf{x}_j - \mathbf{x}_{j'}|^{2m} \exp\{-\beta|\mathbf{x}_j - \mathbf{x}_{j'}|^2\} \quad (7)$$

where m is an integer >0, and β a constant >0. This leads to:

$$\bar{\Phi} = \frac{n(n-1)}{2} \left| \left[\prod_{j'>j}^{n,n} \frac{\partial^m}{\partial \beta_{jj'}{}^m} \left(-H_1 + H_2 \right) \right] \cdot \left[\prod_{j'>j}^{n,n} \frac{\partial^m}{\partial \beta_{jj'}{}^m} D^{-3/2} \right]^{-1} \right|_{\beta_{jj'} = \ldots = \zeta} \quad (8)$$

where $H_1 = C_1(D + 2c_1 \partial D/\partial\beta_{12})^{-3/2}$

$H_2 = C_2(D + 2c_2 \partial D/\partial\beta_{12})^{-3/2}$ and

D is the discriminant of $\sum_{j'>j}^{n,n} \sum \beta_{jj'}(u_j - u_{j'})^2$

The distributions (3), (5) and (7) are of interest as models for the liquid or solid state because they also lead to simple or manageable expressions for the configurational entropy, $S_c = -k\int..\int \rho \ln \rho . d\mathbf{x}_1 .. d\mathbf{x}_n$ and hence to expressions for the free energy which can be minimized (numerically) with respect to the parameters which determine the probability density (that is to say, the $\sigma(\mathbf{k},\alpha)$'s, the $Q_{jj'}^{\alpha\alpha'}$'s and β). Preliminary computations for argon on the basis of (3) have yielded fairly encouraging results; details of the method and results of numerical work based on (3), (5) and (7) will be reported elsewhere.

K. Singer

Department of Chemistry,
Royal Holloway College,
Englefield Green,
Surrey.
Sept. 5.

[1] Domb and Zucker, *Nature*, **178**, 484 (1956).

Improved Resolution of X-Ray Diffraction Patterns of Protein Crystals at Low Temperature

Possibilities for X-ray crystal structure analysis of proteins are limited by the minimum spacings observed in diffraction. For many proteins, the minimum spacing is about 2·5 A.—a limit which does not permit the resolution of individual atoms in Fourier maps prepared from the data, even if these are accurately measured, and the problem of the determination of the phases of the X-ray diffraction spectra can be solved successfully. This lack of resolution puts a severe restriction on the use of Fourier methods for structure determination of proteins. For a few proteins, such as bovine pancreatic ribonuclease, data with a minimum spacing of 1·5 A. are available, so that resolution of atoms is possible in principle. Thus the study of protein structure is highly dependent on fortune in obtaining crystals permitting atomic resolution in their diffraction effects.

An investigation of the causes of the limit on the minimum spacing should be of value in indicating possible ways of improving the situation, as well as in providing information about the nature of protein crystals. In general, the value of the minimum spacing is a measure of the average of the deviations of atoms from their mean positions in the structure; these deviations are due to types of disorder which might be either static or dynamic. Static disorder may appear as a result of heterogeneity (configurational or chemical) of the protein, or as a result of packing faults. Dynamic disorder is likely to be a complex phenomenon in protein crystals, involving restricted Brownian movement of the protein molecules about their mean positions, coupled with the surge of molecules of the solvent from one interstice to the next, in addition to the normal thermal vibration existing within each protein molecule. Brownian movement is likely to be greatest for mechanically weak protein crystals, in accord with experience at the Protein Structure Project with th$_e$

minimum spacings observed for the various modifications of ribonuclease.

If the dynamic disorder is a large component of the total disorder within a protein crystal, the resolution of the diffraction should improve with decrease of temperature, with a limit on improvement, however, imposed by the tendency for 'freezing-in' of disorder at low temperatures, and the fact that the interstices will not, in general, hold an integral number of solvent molecules.

Low-temperature diffraction studies of crystals of ribonuclease II were made by Dr. Vittorio Luzzati (now at Centre de Recherches sur les Macromolecules, 6 rue Boussingault, Strasbourg, France) at the Protein Structure Project; these crystals had been grown from various aqueous alcohols[1]. He found that each crystal showed an abrupt increase in mosaic character at a temperature approximately equal to the freezing point of the pure solvent. This phenomenon is probably caused by disruption of the protein crystal as crystallites of the solvent are produced within the interstices, and precludes, of course, any improvement of resolution on further decrease of temperature.

The range from room temperature to the freezing point of the solvent is usually quite limited. The only recourse appeared then to be to replace the solvent within the interstices with another solution which does not freeze at the desired temperature of study. The investigation of low-temperature diffraction effects of protein crystals was resumed with the study of crystals of ribonuclease II which had been crystallized from 55 vol. per cent 2-methyl-2,4-pentanediol, and had then been soaked for one week in 90 vol. per cent 2-methyl-2,4-pentanediol. The latter composition was chosen as containing the minimum amount of water necessary for maintaining the ribonuclease crystals without visual or X-ray indications of damage. This solvent does not freeze, but becomes highly viscous at dry-ice temperature, and glassy at lower temperatures.

Two crystals were soaked as indicated, mounted in random orientations, and 5° oscillation photographs were taken at room temperature and at −27° C. Of these, the larger crystal showed an improvement at the lower temperature of the minimum observed spacing from 1·69 to 1·41 A., while the smaller showed a change from 1·81 to 1·66 A. On the average, then, the minimum spacing decreased by about 15 per cent. This corresponds to an increase in maximum observed length of reciprocal lattice vector of 15 per cent, or to 50 per cent greater volume covered in reciprocal space.

This degree of improvement of resolution, while not great enough to give a crucial advantage in crystal-structure determination, nevertheless indicates that the limitation on resolution is due at least in part to thermal disorder. It should be noted that ribonuclease crystals give much better resolution than do most protein crystals, and compare well even with simple organic structures, for which the minimum spacing observed is often 0·6–0·7 A. Hence, the room for improvement in resolution in diffraction results from ribonuclease is small. The technique of low-temperature diffraction studies will probably be of more practical benefit in the study of other protein crystals which do not give such good results at room temperature, and may in some cases make possible resolution of atoms in structures for which the room-temperature results do not permit this. In each case, however, it will be necessary to have within the interstices of the crystal a solvent which remains liquid at sufficiently low temperatures. For proteins which would be denatured by organic solvents, solutions of salts or salt mixtures having very low eutectic temperatures might be applicable.

I wish to thank Prof. I. Fankuchen and Dr. Judith Bregman and their co-workers for the use of low-temperature equipment and for invaluable aid and suggestions. Appreciation is expressed to the various institutions (the Dean Langmuir Foundation, the Rockefeller Foundation, the Damon Runyon Fund, New York Foundation, Inc., the Polytechnic Institute of Brooklyn, the International Business Machines Corporation) the continued support of which makes the work of the Protein Structure Project possible.

Murray Vernon King

Protein Structure Project,
Polytechnic Institute of Brooklyn,
55 Johnson St.,
Brooklyn 1, New York.

[1] King, M. V., Magdoff, B. S., Adelman, M. B., and Harker, D., *Acta Cryst.*, **9**, 460 (1956).

Separation of the Lower Fatty Acids (C_1 to C_{10}) by High-Voltage Paper Electrophoresis

Several paper-chromatographic procedures for the separation of the lower fatty acids as their ammonium or ethylamine salts have been described[1]. They suffer, however, from two disadvantages: formic acid cannot be separated from acetic acid and the time required is about 20 hr. By running the chromatograms at 50–60° C. the time required for the separation of the acids C_2 to C_6 could be reduced to 1–2 hr., according to a recent report[2].

During a study of the migration-rates of inorganic cations under high-voltage conditions[3], it was found that under identical conditions many inorganic anions[4] and the lower fatty acids were separable within 10–45 min. The separation of the isomers of C_4 and C_5, however, was not feasible, owing to the very small differences in mobilities. Fig. 1 shows the positions of the acids C_1 to C_{10} under the chosen conditions.

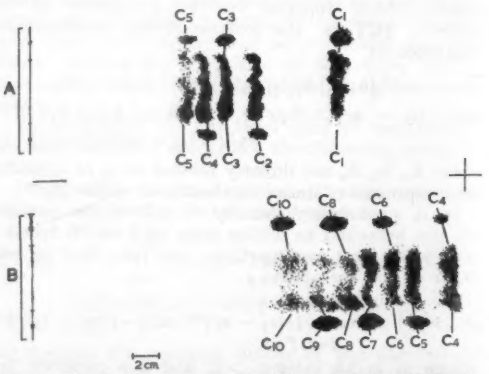


Fig. 1. Electrophoretic separation of the lower fatty acids (C_1 to C_{10}). Conditions: (*A*) 100 V./cm., 13 m.amp./cm., 1·5 lb./in.², Whatman No. 3 *MM*, 4 in. × 22½ in. strip, approx. 0·1 *M* ammonium carbonate solution (7·9 gm./l.), *p*H 8·9, mean temperature of cooling water 12° C., 20 min., applied 2 in. from cathode end 10–20 μgm. of each acid per spot and 50–100 μgm. per band; spraying reagent: 0·025 per cent bromophenol blue in acetone–water (9 : 1) mixture, acidified with 100 mgm. of citric acid per 100 ml. (*B*) as above, 45 min.

The sparingly water-soluble acids C_8 to C_{10} were converted into their sodium salts by dissolution in dilute sodium hydroxide, which did not affect their migration-rates. The acid radical migrates apparently as the free acid, finally forms the ammonium salt and is detected as such.

The first five or six members of the series can be separated within 20 min. at 100 V./cm. (*A*), the rest require 45 min. for complete separation (*B*). The electrograms were dried at 80° C. for about 10 min. and then sprayed with the bromophenol blue reagent, producing blue spots on yellow background stable under ordinary laboratory conditions for at least several hours and of a sensitivity of 2–5 μgm. according to the acid. A more sensitive reagent was found in a solution of 0·05 per cent bromocresol purple in 90 per cent ethanol, with a sensitivity of 0·5–1·0 μgm. for most of the acids but producing rapidly fading colours. The reagent, however, proved useful in the detection of the presence of the faster migrating inorganic anions, for example, chloride and sulphate, in mixtures of fatty acids.

The complete separation of the mixture allowed a convenient quantitative determination of the individual components based on the roughly linear relationship between spot area and concentration over a range of 5–25 μgm. An interesting feature of the paper electrophoretic behaviour of the lower fatty acids is that the mobilities are inversely proportional to the molecular weights, causing a sequence of migration similar to that in vapour-phase chromatography[5] but the reverse of that in paper chromatography. This effect can be utilized for checking doubtful separations by a two-dimensional combination of paper electrophoresis and paper chromatography. There is apparently little correlation between the dissociation constants of the acids and their mobilities, except for the pair formic and acetic acids.

Thanks are due to Mr. H. C. S. de Whalley, director of research, for his interest, Mr. R. W. Butters for technical assistance, and the directors of Tate and Lyle, Ltd., for permission to publish this communication.

D. Gross

Research Laboratory,
Tate and Lyle, Ltd.,
Keston, Kent.
Oct. 29.

[1] Brown, F., and Hall, L. P., *Nature*, **166**, 66 (1950). Hiscox, E. R., and Berridge, N. J., *Nature*, **166**, 522 (1950). Reid, R. L., and Lederer, M., *Biochem. J.*, **50**, 60 (1952).
[2] Roberts, H. R., and Bucek, W., *Anal. Chem.*, **29**, 1447 (1957).
[3] Gross, D., *Nature*, **180**, 596 (1957).
[4] Gross, D., *Chem. and Indust.*, 1597 (1957).
[5] James, A. T., and Martin, A. J. P., *Biochem. J.*, **50**, 679 (1952).

The Basicities of N-Alkyl Anilines

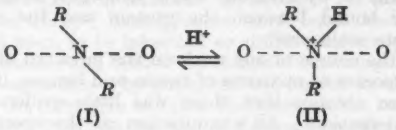
The order of the basicities of the N-alkyl anilines, for which no satisfactory explanation has hitherto been proposed, can be accounted for by a consideration of the intramolecular overcrowding that occurs in the corresponding anilinium ions.

The observed order of basicities[1], measured in water at 25° C. is :

	pK_a		pK_a
Aniline	4·62	Dimethylaniline	5·98
Methylaniline	4·85	Diethylaniline	6·56
Ethylaniline	5·06	Di-*n*-propylaniline	5·59
n-Propylaniline	5·02	Di-*n*-butylaniline	5·7

The order of basicities is usually explained on the grounds of steric inhibition of resonance[2,3], but this fails to account for the large steric requirements of ethyl groups, and indeed has been criticized by Thomson[3].

The results can, however, be explained as follows :



(I) (II)

In the free base (I), the configuration of the molecule is a compromise between the steric requirements of the alkyl groups (*R*) and the coplanarity demanded by conjugation between the amino group and the benzene ring. The bulkier the alkyl groups, the greater the steric inhibition of resonance, and therefore the greater the basicity. On this consideration, pK_a should increase with increase in size of *R*. In the anilinium ion (II), however, conjugation is eliminated, and the configurational requirements of the ion forces one of the alkyl groups towards an *ortho* position. The larger the alkyl group, the greater the resulting instability of the anilinium ion ; thus the basicity will be reduced.

When *R* is a methyl or ethyl group, intramolecular overcrowding in the anilinium ion is small, and the increasing effect of steric inhibition of resonance in the free base gives an increased basicity on passing from *R* = methyl to *R* = ethyl. For the larger *n*-propyl and *n*-butyl groups, however, instability of the anilinium ion causes a fall in basicity, since no further increase in steric inhibition of resonance can occur.

A similar explanation has been given by Brown[4], using the terms '*B*-strain' and '*F*-strain', to account for the reduced basicity of dimethyl-*m*-2-xylidine over dimethyl-*o*-toluidine. In using these concepts to account for the reduced basicity of trimethylamine over dimethylamine, however, Brown[5] is on more difficult ground, since it is not valid[6] to make a direct comparison of the dissociation constants of primary, secondary and tertiary amines.

H. T. Taylor

Dept. of Chemistry and Metallurgy,
Technical College,
Coventry.
Sept. 13.

[1] Hall and Sprinkle, *J. Amer. Chem. Soc.*, **54**, 3469 (1932). Vexlearschi and Rumpf, *C.R. Acad. Sci., Paris*, **223**, 1152 (1949).
[2] Baddeley, *Nature*, **144**, 444 (1939).
[3] Thomson, *J. Chem. Soc.*, 1113 (1946).
[4] Brown and Cahn, *J. Amer. Chem. Soc.*, **72**, 2939 (1950).
[5] Brown, *et al.*, *J. Amer. Chem. Soc.*, **67**, 374 (1945) ; **69**, 1137 (1947).
[6] See Bell and Trotman-Dickenson, *J. Chem. Soc.*, 1289 and 1293 (1949).

Infra-red Absorption Spectra of Casein and Lactose

A communication by Goulden[1], stating that an interaction between protein and sugar is demonstrated by the infra-red absorption spectra of mixtures of the two, prompted us to study this system in the hope that a similar approach might yield information about antibody–antigen reactions.

The spectrum of a dry intimate mixture of casein and lactose shows sharp bands between 7·0 and 11·0μ, whereas the spectrum of a freeze-dried solution of the same mixture shows broad diffuse absorption bands between these wave-lengths. As these differ-

ences occur in a region of the spectrum associated with C—O stretching and O—H bending vibrations, Goulden suggested that the casein and lactose are held together either by some form of hydrogen bonding or by residual water molecules which are firmly bound between the protein and the carbohydrate molecules[2].

In the course of our work on the infra-red absorption spectra of mixtures of casein and lactose, it soon became obvious that there was little evidence for an interaction. An examination of the spectra of α-lactose monohydrate, anhydrous α-lactose, β-lactose and the glasses formed by evaporating aqueous solutions of these sugars showed that the glasses gave spectra with broad, poorly resolved bands, whereas samples of the same sugars examined as finely ground powders in mineral oil or in potassium bromide or chloride disks gave sharper and better resolved absorption bands (Fig. 1). This dependence of the spectrum of a sugar upon the physical state of the specimen has been reported by several workers[3–5]. It seems likely, therefore, that a large part of the difference in spectrum attributed to an interaction between casein and lactose is due to the lactose changing from the crystalline to the glassy (amorphous) state. This possibility is confirmed by two simple experiments. (1) The spectrum of a freeze-dried solution containing both casein and lactose can be reproduced by casting a film of lactose in the glassy state on one side of a silver chloride support and a film of casein on the other. (2) The change observed by Goulden can be reversed by exposing a film of casein and amorphous lactose to a humid atmosphere (relative humidity 75 per cent); under these conditions the amorphous lactose recrystallizes to the α-monohydrate. It may still be that there is some residual change in spectrum due to an interaction, but its magnitude is much less than that due to the change in the lactose.

The infra-red absorption spectra of pressed alkali halide disks containing sugar are liable to change during storage[4], and to minimize such changes we have followed the procedure recommended by Barker *et al.*[5]. Despite these precautions changes have been observed in the spectra of disks stored for several months. In particular, the conversion of anhydrous α-lactose to α-lactose monohydrate and β-lactose to α-lactose monohydrate can be followed by changes in the spectrum of the disk. Specimens of anhydrous α-lactose have to be handled with care because, when they are exposed to the laboratory atmosphere (relative humidity 60 per cent), the change to α-lactose monohydrate takes place in less than three hours.

The physical state of lactose obtained by drying a solution of lactose depends upon the conditions of drying, the presence of materials other than lactose in the solution and their concentrations. The monohydrate of α-lactose crystallizes easily from solution at temperatures below 95° C., and the anhydrous sugar can be prepared by heating the monohydrate at 120–130° C.

The importance of the conditions of drying is indicated by the published spectra[2] of heat-dried full-cream milk and freeze-dried separated milk, which show bands typical of α-lactose monohydrate and the glass form respectively.

We wish to thank Mr. S. J. Lovett for making the sample of β-lactose; this had $[\alpha]_D^{19°} = 34°$.

K. P. Norris
J. E. S. Greenstreet

Microbiological Research Establishment
(Ministry of Supply),
Porton, Near Salisbury,
Wilts.

[1] Goulden, J. D. S., *Nature*, **177**, 85 (1956).
[2] Goulden, J. D. S., *J. Sci. Food Agric.*, **9**, 609 (1956).
[3] Kuhn, L. P., *Anal. Chem.*, **22**, 276 (1950). Thompson, H. W., Nicholson, D. L., and Short, L. N., *Disc. Farad. Soc.*, No. 9, 222 (1950). Barker, S. A., Bourne, E. J., and Whiffen, D. H., "Methods of Biochemical Analysis", **3**, 213 (Interscl. Pub., Inc., New York, 1956).
[4] Barker, S. A., Bourne, E. J., Neely, W. B., and Whiffen, D. H., *Chem. and Indust.*, 1418 (1954). Farmer, V. C., *Spectrochim. Acta*, **8**, 374 (1957).
[5] Barker, S. A., Bourne, E. J., Weigel, H., and Whiffen, D. H., *Chem. and Indust.*, 318 (1956).

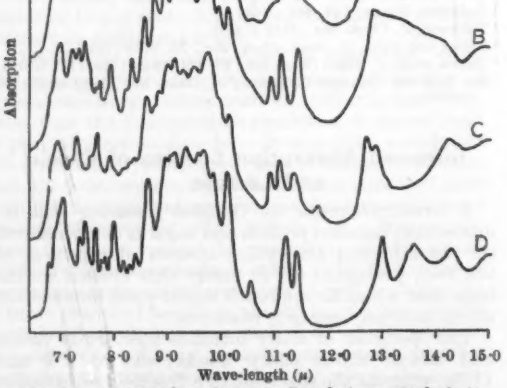


Fig. 1. *A*, Amorphous lactose; *B*, α-lactose monohydrate; *C*, anhydrous α-lactose; *D*, β-lactose. *A* is a glass-like film on silver chloride; *B*, *C* and *D* are potassium chloride disks

Effects of Crystallinity on the Infra-red Absorption Spectra of Lactose and Dried Milk

In an earlier communication[1], it was shown that the infra-red absorption spectra of lactose–casein mixtures showed very marked changes after freeze-drying from aqueous solution. This was interpreted as indicating the presence of an interaction between the lactose and the casein. During the course of attempts to elucidate further the nature of this interaction, it has now been shown that these changes are due largely to the state of crystallinity of the lactose.

Fig. 1*a* shows the spectrum of crystalline lactose, obtained from a sample of the crystalline α-monohydrate. Fig. 1*b* shows the spectrum of amorphous lactose, obtained by freeze-drying a dilute aqueous solution, or by heat-drying a thin film of lactose solution on the surface of a barium fluoride plate. Examination with a polarizing microscope of the samples used for these spectra confirmed the presence of crystalline and amorphous material in the respective samples.

When saturated lactose solutions were used for freeze-drying, the lactose appeared in the crystalline

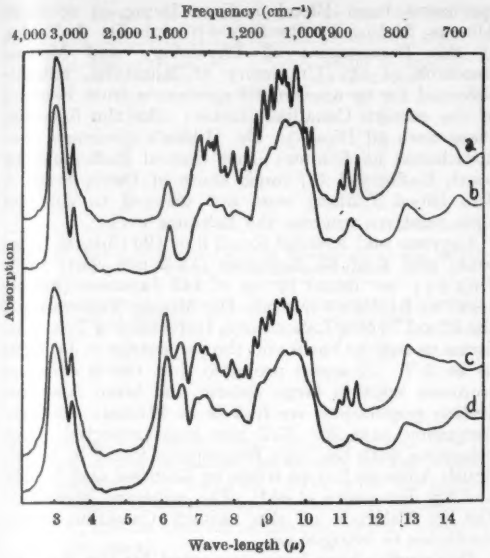


Fig. 1. Infra-red spectra of (*a*) crystalline lactose, (*b*) amorphous lactose, (*c*) 'soluble' dried separated milk, (*d*) dried separated milk

form, if the initial freezing stage was carried out too slowly. The effects of this can be seen in the spectrum 1*A* of ref. 1.

These results are of particular interest in the interpretation of the infra-red spectra of dried-milk samples, since the spectra of samples of dried separated milk made 'soluble' by the 'Instant' process[2] show that the lactose has been converted into the crystalline form (Fig. 1*c*). The spectra of samples which have been dried in the normal way show that the lactose exists in the amorphous form (Fig. 1*d*). Further confirmation of this change in the state of the lactose is afforded by freeze-drying solutions of 'soluble' milk powder, when the spectra are similar to Fig. 1*d*, showing that the lactose has reverted to the amorphous form.

The conversion of dry amorphous lactose to the crystalline α-monohydrate on exposure to atmospheric water vapour can be shown by the infra-red spectra, and similar changes can also be seen in the spectra of dried-milk samples under similar conditions.

The spectra were obtained with a double-beam Grubb Parsons *S3A* spectrometer fitted with a rock-salt prism, and the samples were examined using the potassium bromide pressed-disk technique. Similar results were obtained when the samples were examined by the 'Nujol' mull technique.

J. D. S. Goulden

Physics Department,
National Institute for Research
in Dairying,
University of Reading.

Jonathan W. White, jun.

Eastern Utilization Agricultural
Research Service,
Research and Development Division,
United States Department
of Agriculture,
Philadelphia 18, Pennsylvania.

[1] Goulden, J. D. S., *Nature*, **177**, 85 (1956).
[2] Havighorst, C. R., *Food Eng.*, **26**, 74 (1954).

'Multiple' Hæmoglobins in the Horse

The finding of electrophoretically distinct hæmoglobins in man has, in recent years, been followed by similar reports in animals. In cattle[1,2], for example, and in sheep[3], hæmoglobins have been discovered which seem to be inherited as simple allelomorphs.

Cabannes and Serain[1] examined the hæmoglobin of forty-eight horses by paper electrophoresis at alkaline *p*H. In forty-five samples the hæmoglobin resolved into two fractions moving at different speeds; in the remaining three only the faster-moving component was seen. We have examined hæmoglobin from sixty-five horses and found two fractions in sixty-four and only one, the fast-moving component, in one. Thus of 113 samples, 109 (96·5 per cent) were composed of two components, and four (3·5 per cent) showed only one, which was the fast-moving component in each.

If, as in cattle and sheep, the two hæmoglobins were allelomorphs, this result would represent a remarkable example of hybrid vigour. We decided, therefore, to re-investigate the one horse in which we had found only one hæmoglobin. By the use of starch or agar zone electrophoresis, in which larger quantities of protein can be used, it was found that this specimen also consisted of two hæmoglobin fractions. But, whereas usually the quantity of slow to fast is 0·67 : 1, in this particular horse it was 0·25 : 1. By using thicker paper and more hæmoglobin, the slow fraction could be demonstrated also by paper electrophoresis.

We were thus satisfied that in our series of sixty-five specimens two fractions were present in all, and that this distribution strongly indicated the presence of 'multiple' hæmoglobins in the horse rather than that of two allelomorphs.

We have another indication that the two horse hæmoglobins are not allelomorphs, as it seems that mules and jennets do not inherit one but both these hæmoglobins. Cabannes and Serain[1] had shown that donkeys possess one hæmoglobin and that mules may possess two. Of four mules, two showed a hæmoglobin pattern not unlike that of the horse, and in two, paper electrophoresis demonstrated only one, the slow-moving fraction. In fourteen specimens from donkeys, we have found only one fraction which moved slightly faster than the 'slow' horse fraction. On examining blood from nineteen mules and three jennets, each one was found by us to be resolved into two fractions, of which the faster amounted to about 38 per cent; neither fraction moved exactly in coincidence with those of the horse hæmoglobin (Fig. 1). When donkey hæmoglobin

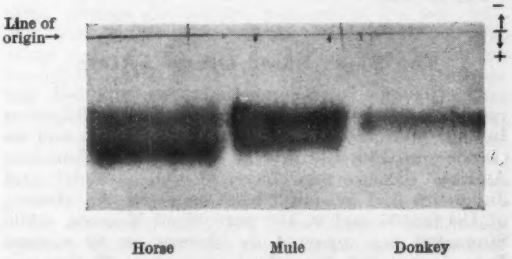


Fig. 1. Paper electrophoresis at *p*H 8·6 of horse, mule and donkey hæmoglobin ('hanging strip' technique, ref. 4). Compared with the two fractions of the horse, the 'slow' component of the mule hæmoglobin moves a little faster, and the 'fast' component more slowly. The single band seen in the donkey hæmoglobin does not coincide with any of the other four

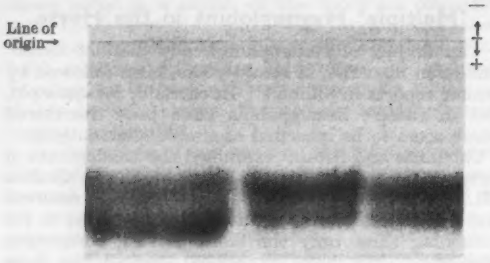


Fig. 2. Comparison by paper electrophoresis at pH 8·6 of the hæmoglobin of horse and mule, and of a mixture from horse and donkey ('hanging strip' technique, ref. 4). The mixture of horse and donkey hæmoglobins resemble mule hæmoglobin in its electrophoretic behaviour. The 'fast' component of the horse pigment moves in this mixture like the 'fast' component in the mule

was added to horse hæmoglobin so that the proportion of the 'fast' horse component was 38 per cent of the total, then the mixture showed an electrophoretic pattern identical with that of mule or jennet hæmoglobin (Fig. 2). When the fast-moving fraction of mule hæmoglobin or of the donkey/horse mixture was eluted and added to an eluted solution of 'slow' horse hæmoglobin, this mixture produced a pattern identical with that found in horse. Although we have not achieved a resolution of mule and jennet hæmoglobin into three fractions (one donkey and two horse hæmoglobins), it seems to us that there is circumstantial evidence that three hæmoglobins are in fact present.

We wish to acknowledge the help of the following in obtaining samples of blood: Dr. R. K. Archer, Equine Research Station, Newmarket; Capt. H. W. Bishop, R.A.V.C., Hong Kong; Dr. S. L. Hignett and Mr. J. Scarnell, Wellcome Veterinary Research Station, Frant; Prof. J. A. Nicholsen, Veterinary College of Ireland, Dublin; and Mr. D. Russel, Tipperary.

A. D. Bangham

Agricultural Research Council,
Institute of Animal Physiology,
Babraham Hall,
Babraham, Cambridge.

H. Lehmann

St. Bartholomew's Hospital,
London, E.C.1.
Oct. 4.

[1] Cabannes, R., and Serain, C., *C.R. Soc. Biol., Paris*, **149**, 1193 (1955).
[2] Bangham, A. D., *Nature*, **179**, 467 (1957).
[3] Evans, J. V., King, J. W. B., Cohen, B. L., Harris, H., and Warren, F. L., *Nature*, **178**, 849 (1956).
[4] Lehmann, H., and Smith, E. B., *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, 12 (1954).

The Diego Blood Group System

In previous communications we reported the presence of the blood group antigen *Di*[a] in Chippewa Indians in North America and in Japanese[1], and its absence in 156 Eskimos of the eastern Canadian Arctic[2]. Since then Layrisse and Arends[3] and Junqueira and Wishart[4] have reported the absence of *Di*[a] in 107 and in 150 pure blood Negroes, while Simmons[5] has reported its absence in 80 eastern Polynesians, 162 Australian aborigines, 23 Papuans and 74 natives of New Britain.

We have now had an opportunity of studying, through the kindness of Dr. Eric Nation of the Canadian Red Cross Blood Transfusion Service, 50 specimens from Blood Indians living in southern Alberta, finding 3 to be *Di*(a+), while Dr. J. Hildes, of the Department of Physiology and Medical Research of the University of Manitoba, recently collected for us another 68 specimens from Eskimos of the eastern Canadian Arctic; like the first 156, these were all *Di*(a−). Dr. Hildes's specimens were distributed as follows: east central Baffinland 49, south Baffinland 11, south shore of Davis Strait 8. The Blood Indians were not selected to rule out close relatives whereas the Eskimos were.

Layrisse and Arends[6] found 5 of 100 Chinese (5 per cent) and 8 of 65 Japanese (12·3 per cent) to be *Di*(a+); we[7] found 10 out of 145 Japanese (6·9 per cent) to be *Di*(a+), while Dr. Mitsuo Yokayama of the Blood Typing Laboratory, University of Tokyo, informs us that he has found the percentage in Japanese to be 5–7. It seems probable that *Di*[a] is about as common both in large Asiatic and large American Indian populations as *Kell* is in Whites; no high-frequency area for *Kell* has been reported which compares with the high frequencies found in certain South America Indian tribes by Layrisse and Arends[8] and by Junqueira *et al.*[9]. The apparent absence of *Di*[a] in Eskimos of the eastern Canadian Arctic continues to intrigue us.

Our studies have been supported by the National Museum of Canada.

Bruce Chown
Marion Lewis
Hiroko Kaita

Blood Group Reference and
Research Laboratory,
735 Notre Dame Ave.,
Winnipeg 3, Manitoba.

[1] Lewis, M., Ayukawa, H., Chown, B., and Levine, P., *Nature*, **177**, 1084 (1956).
[2] Lewis, M., Chown, B., and Kaita, H., *Nature*, **178**, 1125 (1956).
[3] Layrisse, M., and Arends, T., *Nature*, **179**, 478 (1957).
[4] Junqueira, P. C., and Wishart, P. J., *Nature*, **180**, 341 (1957).
[5] Simmons, R. T., *Nature*, **179**, 1352 (1957).
[6] Layrisse, M., and Arends, T., *Nature*, **177**, 1083 (1956).
[7] Lewis, M., Kaita, H., and Chown, B., *Amer. J. Human Genet.* (in the press).
[8] Layrisse, M., and Arends, T., *Science*, **123**, 633 (1956).
[9] Junqueira, P. C., Wishart, P. J., Ottensooser, F., Pasqualin, R., Loureiro Fernandez, P., and Kalmus, H., *Nature*, **177**, 41 (1956).

Separation of Carotenoid Synthesis from Fat Synthesis in *Rhodotorula gracilis* by Diphenylamine

In the fat-synthesizing red yeast *Rhodotorula gracilis* Rennerfelt fat synthesis occurs simultaneously with carotenoid synthesis. Since Turian[1] and Goodwin[2] found that carotenogenesis in *Mycobacterium phlei* and in *Phycomyces blakesleeanus* can be inhibited by diphenylamine, we thought it of interest to see whether carotenoid and fat synthesis in *R. gracilis* might be separated by the action of diphenylamine.

The cells of *R. gracilis* were grown aerobically at 25° C. for three days in a synthetic medium[3] containing various amounts of diphenylamine. The concentrations of diphenylamine tested and the final dry-weight determinations of the cells are given in Table 1. The results show that with up to 45 mgm. of diphenylamine in 1,000 ml. of the cultivation medium there is no inhibition of growth of *R. gracilis* to be noted, although it could be observed that the colour of the cells diminished gradually with increasing concentrations of diphenylamine. With 40 and 45 mgm. diphenylamine in 1,000 ml. medium, the cells are colourless and do not contain any

Table 1. Effects of Diphenylamine on the Growth of *Rhodotorula gracilis*

Mgm. diphenylamine in 1,000 ml. medium	Dry weight of cells in 10 ml. medium
0	71·2
5	70·5
10	70·8
20	71·0
30	70·2
40	69·8
45	69·5
50	1·2

Table 2. Fat Content of Cells of *Rhodotorula gracilis*, grown in the Presence of Diphenylamine

Mgm. diphenylamine in 1,000 ml. medium	Per cent fat in the dry weight of the cells
0	60·5
5	61·0
30	59·8
45	57·0

carotenoids. In these conditions, however, the fat synthesis is not impaired, as shown by fat content determinations (cf. Table 2). Even with the highest concentration of diphenylamine, namely, 45 mgm. in 1,000 ml., the fat content of the cells is only insignificantly decreased.

In agreement with Goodwin[2], we found the inhibitory action of diphenylamine towards carotenogenesis to be irreversible in a certain sense. When the cells of *R. gracilis* are grown in a medium containing diphenylamine and then, after centrifugation and washing, are transferred into a medium which contains neither diphenylamine nor a nitrogen source, where under normal conditions carotenoid synthesis can take place, the cells remain colourless. If, however, the cells treated with diphenylamine are transferred into a medium containing a nitrogen source and thus are allowed to proliferate, they gradually recover their capacity of carotenoid synthesis.

L. Šlechta
O. Gabriel
O. Hoffmann-Ostenhof

Erstes Chemisches Institut
der Universität,
Vienna. Oct.9.

[1] Turian, G., *Helv. Chim. Acta*, **33**, 1988 (1950).
[2] Goodwin, T. W., Jamikorn, M., and Willmer, J. S., *Biochem. J.*, **53**, 531 (1953).
[3] Kleinzeller, A., Málek, J., Praus, R., and Škoda, J., *Chem. Listy*, **46**, 470 (1952).

Preparation of Transparent Starch Gels after Electrophoresis

In the method of electrophoresis on starch gel recommended by Smithies[1], the gels obtained after the electrophoresis and staining of proteins are opaque, difficult to preserve and not suitable for photometric evaluation. For this reason we have devised a technique for preparing starch gels which remain transparent after staining.

Since a protein dye in alcoholic solution, for example, a saturated solution of amidoschwarz in 10 per cent acetic acid in methanol, results in a permanently opaque gel, we prefer to use the dye in aqueous solution; 1 per cent amidoschwarz in a sodium acetate/acetic acid buffer (sodium acetate 0·1 *M*, 450 ml., acetic acid *M*, 450 ml., amidoschwarz, 1 gm.), being used for staining in agar electrophoresis[2].

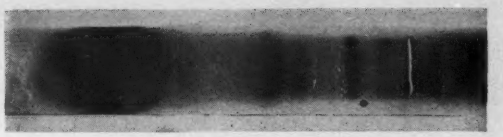


Fig. 1. Transparent starch gel after electrophoresis (human serum)

After fixing for one night in 5 per cent aqueous acetic acid the bands are stained for 10 min. and decolorized by successive washings in 5 per cent aqueous acetic acid. This washing should be prolonged (nearly 48 hr.).

A completely transparent starch gel can be obtained in the following way. A 1-mm. transverse section of stained starch gel is placed on a plate of photographic glass which has been covered with a film of melted agar and then dried in an oven. The glass plate is placed in a rectangular basin and a 1·50 per cent solution of melted agar is poured over it. After the agar has solidified the glass plate carrying the starch electrophoresis included in agar is withdrawn and a sheet of paper (Arches 302 or Whatman No. 1), is applied to the surface so as to regularize the evaporation and to absorb the non-volatile mineral salts. It is left to evaporate in an oven at 37° C. After 48 hr. a transparent film is obtained permitting the photometric evaluation of the different protein fractions of the serum (Fig. 1).

J. M. Fine
E. Waszczenko-Z

Centre National de Transfusion Sanguine,
Paris.

[1] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[2] Uriel, J., and Grabar, P., *Ann. Inst. Pasteur*, **90**, 427 (1956).

Reversal of Thymine Xyloside Inhibition in *Escherichia coli* by Glutathione

An unexpected competitive relationship between thymine xyloside and glutathione has been found. Thymine xyloside inhibits the growth of *Escherichia coli* in a synthetic medium. This inhibition is competitively reversed by glutathione.

A wild-type *E. coli* (ATCC 26) was grown in brain-heart infusion broth for 6 hr. at 37° C., then diluted to 1/50,000 in a synthetic medium of the following composition: sodium phosphate 0·6 per cent, acid sodium phosphate 0·3 per cent, ammonium chloride 0·1 per cent, sodium chloride 0·1 per cent, magnesium sulphate 0·01 per cent, potassium chloride 0·3 per cent, glucose (autoclaved separately) 0·4 per cent. The size of the inoculum in these conditions was about 5×10^4 cells per ml. The inoculated medium was pipetted into sterile 13 mm. × 100 mm. test tubes and the thymine xyloside or reversing agents added in seeded synthetic medium. The tubes were incubated overnight at 37° C., and the presence or absence of growth was determined by visual inspection. The minimum concentration of inhibitor to prevent growth completely was recorded.

The minimum inhibitory concentration of thymine xyloside varied with the preparation used. The best preparation gave an end-point of 1 μgm./ml. in these conditions. However, the reversal experiments were carried out with a preparation of inhibitor which gave an end-point of 6·4 μgm./ml. and were confirmed with the more active material.

Table 1. Growth Inhibition of *E. coli* by Thymine Xyloside and its Reversal by Glutathione

Thymine xyloside concentration (μgm./ml.)	Glutathione concentration necessary to reverse inhibition (μgm./ml.)	Ratio
100	12·3	8/1
33	3·2	10/1
10	0·8	12/1

In a search for a possible reversing agent, glutathione was tried. This proved extremely effective in reversing the inhibition. About 0·1 mole of glutathione reversed 1 mole of thymine xyloside and this reversal was competitive (Table 1).

Reversal studies with purine and pyrimidine bases and nucleosides revealed no significant effects of these compounds. Fair reversal was obtained with technical casamino-acids ('Difco'). A survey of amino-acids revealed no single one which was as active as the casamino-acids. No agent was found which was as effective as glutathione in reversing the inhibition.

This competitive relationship between thymine xyloside and glutathione is quite interesting, and there is no apparent simple biochemical explanation for it. Beltz and Visser[1] have commented that 5-substituted deoxyribosyl-pyrimidines are the only substituted nucleosides which inhibit the growth of micro-organisms not requiring purines and pyrimidines for growth. Since thymine xyloside is a 5-substituted nucleoside with an unnatural sugar, it may be inhibiting in an analogous manner. It might be possible to use thymine xyloside to induce glutathione deficiencies so that glutathione metabolism and function can be more readily studied.

The thymine xyloside used in these studies was prepared by the method of Fox *et al.*[2] and was kindly supplied by Dr. J. H. Hunter of these Laboratories. Dr. Seymour Cohen has given helpful advice and encouragement.

Thomas D. Brock*

Research Laboratories,
The Upjohn Co.,
Kalamazoo, Michigan.

* Present address: Department of Biology, Western Reserve University, Cleveland 6, Ohio.

[1] Beltz, R. E., and Visser, D. W., *J. Amer. Chem. Soc.*, 77, 736 (1955).
[2] Fox, J. J., Yung, N., Davoll, J., and Brown, G. B., *J. Amer. Chem. Soc.*, 78, 2117 (1956).

Aerobic Inhibition of Glycolysis

During investigations on the synthesis of sucrose from glucose-1-phosphate and fructose by pea seed extracts it was observed that a glycolytic system was operating in the enzymic digests[1,2]. The pea extract had been partially purified by precipitation with ammonium sulphate (80 per cent saturation) and dialysis against phosphate buffer at *p*H 7·0. It was found that there were differences in glycolytic activity when the enzymic digests were incubated under aerobic and anaerobic conditions.

When digests containing glucose-1-phosphate and fructose were incubated in air there was a small increase in inorganic phosphate after 40 hr. (Table 1). In nitrogen the inorganic phosphate decreased. Visual examination of the phosphoric compounds of the digest samples on paper chromatograms developed in *n*-propyl alcohol/ammonia/water (60 : 30 : 10, v/v) and *tert*-amyl alcohol/water/formic acid (90 : 90 : 30, v/v)[3] confirmed this decrease in inorganic phosphate and showed that extra phosphoric esters

Table 1. Effect of Anaerobic Conditions on Inorganic Phosphate Content

Gas phase	Inorganic phosphate (μmoles/ml. digest)	
	0 hr.	40 hr.
Air	9·5	11·5
Nitrogen	9·5	2·2

Digest: glucose-1-phosphate, 0·0144 *M*; fructose, 0·0244 *M*; $MgCl_2$, 0·01 *M*; yeast nucleotide fraction, 0·5 ml.; partially purified pea extract, 1 ml.; total volume, 1·8 ml. *p*H 7·0, 25° C.

Table 2. Effect of Anaerobic Conditions on Fructose Loss

Gas phase	Fructose (μmoles/ml. digest)	
	0 hr.	20 hr.
Air	24·4	10·1
Evacuated	24·4	0

Digest: as in Table 1.

accumulated in nitrogen. This accumulation appeared to be largely due to increases in fructose-1,6-diphosphate, hexose monophosphates and a substance running in a position similar to adenosine triphosphate. Incubation of the enzymic digests *in vacuo* gave similar results to those obtained in nitrogen.

Anaerobic conditions also accelerated the decrease in fructose in the enzymic digests (Table 2). Fructose had disappeared after 20 hr. under anaerobic conditions, whereas in air a considerable quantity of this sugar remained.

When the gas exchange of the enzymic digests was examined, it was found that in air there was a barely significant oxygen uptake suggesting that oxidative processes were not proceeding to an appreciable extent. There was a considerable carbon dioxide output in air, and this was increased markedly in an atmosphere of nitrogen.

The effect of oxygen on the enzymic digests may be analogous to the Pasteur effect, for which a number of explanations have been proposed[4–6]. If so, the results obtained in the present investigation may support the hypothesis of Lipmann[7] that oxygen inhibits glycolysis by inactivating one or more of the glycolytic enzymes. In the simple system investigated here (which is not complicated by the existence of respiratory processes) glycolysis is the only source of adenosine triphosphate or energy-rich phosphate bond. It seems probable that the rate of hexose utilization is decreased under aerobic conditions because of a decreased production of adenosine triphosphate and this may in part be due to a partial inactivation of one of the glycolytic enzymes.

J. F. Turner*

(Broodbank Fellow of the University of Cambridge)
Botany School,
Cambridge.

L. W. Mapson

Low Temperature Station for
Research in Biochemistry and Biophysics,
University of Cambridge and Department
of Scientific and Industrial Research.

* Present address: Division of Food Preservation and Transport, C.S.I.R.O., Botany School, University of Sydney.

[1] Turner, J. F., *Nature*, 172, 1149 (1953); 174, 692 (1954).
[2] Turner, J. F., *Biochem. J.* (in the press).
[3] Hanes, C. S., and Isherwood, F. A., *Nature*, 164, 1107 (1949).
[4] Dickens, F., "The Enzymes", 2 (1), 114 (Academic Press, 1951).
[5] Turner, J. S., "Ann. Rev. Plant Physiol.", 2, 145 (1951).
[6] Krebs, H. A., *Endeavour*, 26, 125 (1952).
[7] Lipmann, F., "Symposium on Respiratory Enzymes", 48 (University of Wisconsin Press, 1942).

Formation of Histamine-containing Dinucleotides by Enzymatic Interaction of Histamine and Coenzyme I

DIPHOSPHOPYRIDINE nucleotide (coenzyme I) reacts, in the presence of a soluble purified preparation of diphosphopyridine nucleotidase[1], with 4-amino-5-carboxamido-imidazole yielding the pyrophosphate of adenosine-5′-phosphate and of the 4-amino-5-carboxamido-imidazole ribotide[2,3]. The latter may serve as a precursor in the biosynthesis of inosinic acid[4].

It was of interest to study the influence of various side-chains attached to carbons number 4 and/or 5 upon the ability of the imidazole to participate in such diphosphopyridine nucleotidase catalysed reactions. Histamine was selected as a first example.

In a typical experiment (Table 1) histamine (252 μmol.), diphosphopyridine nucleotide and diphosphopyridine nucleotidase (21 units[1]) were incubated in a volume of 0·25 ml. at 38° C. at an initial *p*H about 8·0 for 4 hr. The *p*H was maintained relatively constant by means of the proper ratio of histamine (free base, 157 μmol.) to histamine dihydrochloride (95 μmol.). This ratio was calculated from the *pK* values of the histamine mono- and di-hydrochlorides (9·7 and 6·0 respectively). Sample number 3 (Table 1) was buffered with *tris*-hydroxy-methyl-amino-methane to *p*H 8·0.

Normally, two molecules of *d*-ribose and two molecules of organically bound phosphate[5] correspond to every coenzyme I molecule, measured by means of the alcohol dehydrogenase[6] and the cyanide[7] techniques. It was found that the amount of *d*-ribose, as measured by conversion to furfural in the presence of orcinol[8], diminished as the incubation proceeded, and tended to reach one-half of its original value (Table 1). However, periodate oxidation used for quantitative determination of vicinal *cis*-hydroxyls (*cis*-glycols)[9], represented by the 2,3-alcoholic hydroxyls of the *d*-ribose, indicated that their number per mol. of coenzyme I remained constant during incubation and was the same as the number of organically bound phosphate groups (Table 1).

In order to separate the products of this reaction, coenzyme I (340 μmol.) and diphosphopyridine nucleotidase (280 units) were incubated with carbon-14 2-(ring)-labelled histamine (500 μc., 2,000 μmol. of the described mixture of the free base and its dihydrochloride). The initial *p*H was approximately 8·0 and the total volume was 4 ml. Upon consumption of all the coenzyme I present (16 hr., 38° C.), the mixture was passed through a 'Dowex' 1 × 10, chloride form, column.

The main product of the reaction, a dinucleotide (dinucleotide I), was eluted with 0·00025 *N* hydrochloric acid, *p*H 3·6. A second dinucleotide (dinucleotide II) was eluted from the column with 0·004 *N* hydrochloric acid, *p*H 2·4, in much smaller quantities (approximately a quarter of dinucleotide I).

Both dinucleotides I and II corresponded to the formula: Histamine–(ribose)–phosphate–phosphate–ribose–adenine. For as yet unknown reasons, the ribose moiety placed in the formula in parenthesis was not reactive in the orcinol test[8]. Both dinucleotides I and II were radioactive (about 0·24 μc. per μmol.), gave positive ninhydrin tests (primary amines), and crystallized as picrates. They coupled with diazotized sulphanilic acid (yellow at *p*H 10, maxima at 402 mμ and 438 mμ respectively), and had characteristic absorption spectra in the ultra-violet. For example, at *p*H 2·0, dinucleotide I showed two maxima, at 257 mμ and 208 mμ, and a minimum at 234 mμ. Dinucleotide II at the same *p*H, besides the maximum at 257 mμ, had a shoulder at about 208 mμ. Organic pyrophosphatase[10] split both dinucleotides I and II into adenosine-5′-phosphate[3] and a histamine–(ribose)–phosphate moiety (mononucleotides I and II). Ascending R_F values in the system alcohol/0·1 *M* acetic acid (1 : 1)[4] were 0·53 and 0·68 for dinucleotides I and II respectively; 0·69 and 0·76 for mononucleotides I and II respectively.

Electrophoretic studies[3] were conducted in ammonium formate, *p*H 3·6 and *tris*-hydroxy-methyl-amino-methane, *p*H 7·6, at concentrations that showed specific resistance of 210 ohms at 4° C. and potential differences of 10 volts per cm. At *p*H 7·6 dinucleotide I and mononucleotide I moved towards the positive electrode and covered the same distance as coenzyme I, while dinucleotide II and mononucleotide II covered about twice the distance in the same direction. The mobility of the latter compounds was similar to that of adenosine-5′-phosphate at the same *p*H. At *p*H 3·6, dinucleotide I had zero mobility, while mononucleotide I moved towards the negative electrode. Dinucleotide II moved towards the positive electrode and mononucleotide II had zero mobility.

From the analytical results and the action of organic pyrophosphatase, it was concluded that dinucleotides I and II, like coenzyme I, possessed two negative charges at both *p*H 3·6 and 7·6, and mononucleotides I and II, like adenosine-5′-phosphate, possessed two negative groups at *p*H 7·6, and one at *p*H 3·6. Therefore, the above-mentioned mobilities might indicate that dinucleotide I had a pentavalent nitrogen structure which retained its positive charge at both *p*H 3·6 and 7·6 while dinucleotide II lacked such a structure. Both dinucleotides I and II had an additional positive group fully ionized at *p*H 3·6 (probably the aliphatic amine). The presence of a pentavalent nitrogen structure in dinucleotide I and of a trivalent nitrogen structure in dinucleotide II indicated a combination of either the basic or the pseudo-acidic forms of the imidazole[11] with (ribose). This may have resulted from changes in the *p*H

Table 1

Sample	Coenzyme I				Organic phosphate		Periodate consumed		*d*-Ribose	
	Alcohol dehydrogenase		Cyanide							
	Zero time	4 hr.	Zero time	4 hr.	Zero time	4 hr.	Zero time	4 hr.	Zero time	4 hr.
1 Complete system	4·90	0·00	5·20	0·42	11·30	11·40	12·60	12·80	10·70	6·60
2 Coenzyme I omitted	0·02	0·00	0·08	0·04	0·84	0·90	2·60	2·80	0·25	0·47
3 Histamine omitted	4·30	0·00	4·50	0·38	9·50	10·00	11·70	12·90	9·26	10·10
4 Enzyme omitted	4·60	4·22	5·00	4·44	10·30	10·60	10·10	10·20	9·70	9·90

All figures in μmoles.

(from *p*H 8·0 to about 6·0) during the enzymatic reaction. Such *p*H changes can be tolerated by the enzyme[1,12].

Under our conditions the enzymatic reaction appeared to proceed completely towards the formation of the new dinucleotides. Nicotinamide inhibited this reaction.

Since histamine may be released in tissues[13] and in view of the wide distribution of diphosphopyridine nucleotidases of the glycosidase type similar to the one used in these experiments, it is possible that the reaction described may be involved in the mechanism of anaphylactic shock. Experiments are under way to test this possibility.

I am indebted to Dr. Israel Davidsohn, director of research, and Dr. Kurt Stern, associate director of Mount Sinai Medical Research Foundation, Chicago, for their interest in this work.

Spyridon G. A. Alivisatos

The Mount Sinai Medical
Research Foundation,
Chicago, Illinois. July 27.

[1] Alivisatos, S. G. A., and Woolley, D. W., *J. Biol. Chem.*, **219**, 823 (1956).
[2] Alivisatos, S. G. A., and Woolley, D. W., *J. Amer. Chem. Soc.*, **77**, 1065 (1955).
[3] Alivisatos, S. G. A., and Woolley, D. W., *J. Biol. Chem.*, **221**, 651 (1956).
[4] Alivisatos, S. G. A., Proc. Canad. Physiol. Soc., 20th Ann. Meeting, 4 (1956).
[5] King, E. J., *Biochem. J.*, **26**, 292 (1932).
[6] Zatman, L. J., Kaplan, N. O., and Colowick, S. P., *J. Biol. Chem.*, **200**, 197 (1953).
[7] Colowick, S. P., Kaplan, N. O., and Ciotti, M. M., *J. Biol. Chem.*, **191**, 447 (1951).
[8] Mejbaum, W., *Z. physiol. Chem.*, **258**, 117 (1939).
[9] Dixon, J. S., and Lipkin, D., *Anal. Chem.*, **26**, 1092 (1954).
[10] Kornberg, A., and Pricer, jun., W. E., *J. Biol. Chem.*, **182**, 763 (1950).
[11] Hofmann, K., "Imidazole and its Derivatives", Pt. 1, 14 (Interscience Pub., 1953).
[12] Alivisatos, S. G. A., Kashket, S., and Denstedt, O. F., *Canad. J. Biochem. Physiol.*, **34**, 46 (1956).
[13] Paton, W. D. M., *Int. Arch. Allergy*, **6**, 203 (1955).

Accumulation of α-Alanine and of Pyruvate in Diphtherial Culture Filtrate and its Relation to Iron Deficiency of Medium

It was shown by Linggood and Woiwod[1] that marked changes, particularly an increase in the quantity of α-alanine, occurred in the chromatograms of a casein hydrolysate medium before and after the growth of a strain of *C. diphtheriae* under conditions favourable for toxin production. While studying the effect of iron on the amino-acid metabolism of a strain of *C. diphtheriae* in a simplified, chemically defined medium developed in our laboratory[2,3], we have also found that several amino-acids, especially α-alanine, appear and accumulate in the culture filtrate when the medium is deficient in iron. In further experiments, it has also been demonstrated that an accumulation of pyruvate takes place under the same conditions, and that the amounts of α-alanine and pyruvate appearing in the filtrates, decrease with the increasing iron content of the medium.

Small inocula of the four-day-old pericle of a variant (*SM*–1) of Park Williams No. 8 strain were grown on AMC media[2] containing varying amounts of iron. After four days of still incubation, the whole cultures were harvested and centrifuged at 4,000 r.p.m. for 30 min. The precipitated cells were washed three times with saline before measuring the bacterial nitrogen by a

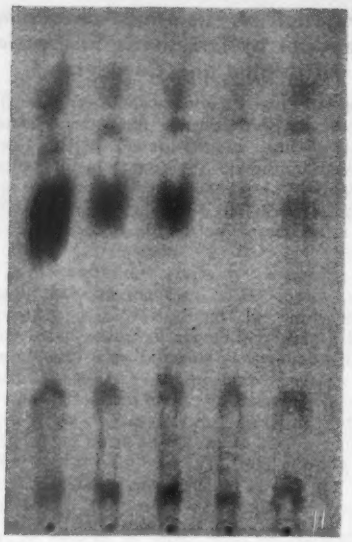


Fig. 1. One-dimensional paper chromatogram of supernatants harvested from diphtherial cultures grown with varying amounts of iron. 1, Aspartic acid and lysine group; 2, glutamic acid and cystine group; 3, asparagine, glycine and serine group; 4, α-alanine; 5, valine; 6, methionine, leucine and isoleucine group. μgm. of iron added per litre of medium: *A*, none; *B*, 66; *C*, 200; *D*, 330; *E*, 1,000. Solvent used: phenol/water (4 : 1 v/v)

modified micro-Kjeldahl method, while the clear supernatants were used as materials for paper chromatography and also for the estimation of toxin by flocculation reactions, and of the pyruvate by the method of Friedmann and Haugen[4]. For the quantitative determination of α-alanine, the following method was used: Tōyō filter paper No. 50 was spotted with an aliquot of the supernatant, which had been boiled at *p*H 4·6, and then developed with phenol/water (4 : 1 v/v) and butanol/acetic acid/water (4 : 1 : 1 v/v/v) by the ascending technique. The part of the paper corresponding to the location of α-alanine on the two-dimensional chromatogram was cut to pieces and placed in a test tube. After complete colour development according to Fowden[5], the disrupted papers were removed by centrifugation at 3,000 r.p.m. for 2 min. and the colour intensity of the clear supernatant was then measured at 570 mμ with a Coleman-type spectrophotometer.

From the accompanying photograph, showing a typical one-dimensional paper chromatogram of the culture filtrates, no major differences can be seen in the amino-acids present in each filtrate; however, the quantity of each amino-acid, particularly of α-alanine, varies with the amount of iron added to the medium. Quantitative results, showing the effect of iron on both α-alanine and pyruvate accumulations, are given in Table 1. Although the medium used in this experiment was not completely free of iron, added iron had a remarkable effect on the formation of α-alanine and of pyruvate by the diphtheria bacillus. It can be seen in Table 1 that the amounts of both α-alanine and pyruvate detected in culture filtrates at the end of incubation markedly decrease with the increase of iron added. Thus, for example, the quantity of pyruvate produced by the organism in the presence of 330–660 μgm./l. of added iron is

Table 1. Effect of Iron on the Formation of α-Alanine and Pyruvate by a Strain of *Corynebacterium diphtheriae*. Four-day Still-incubation at 32–33° C.

Iron added (μgm./l.)	Bacterial growth (μgm. N/ml.)	Toxin (Lf./ml.)	Pyruvate formed (μgm./ml.)	Alanine formed (μgm./ml.)
0	543	60	220	617
16·5	592	55	196	547
33	561	52	172	553
66	634	44	95	373
100	627	47	120	313
133	561	23	57	307
330	546	3	11	127
660	563	0	11	97

almost one-twentieth of the quantity formed in the absence of iron. A similar variation will be seen in the amounts of toxin and α-alanine formed.

Although the primary metabolic sequence affected by iron deficiency still remains uncertain, these results clearly indicate that traces of iron exert a distinct influence not only on the liberation of toxin but also on the pyruvate metabolism of the organism. Our previous work[6] has shown that significant quantities of glutamate–pyruvate transaminase were present in the cell-free extracts of *SM*–1 culture. Consequently, the accumulation of pyruvate due to iron deficiency may result in the appearance of large amounts of α-alanine in the filtrates. Further work is now in progress, and the details will be published elsewhere.

Masahiko Yoneda

Department of Microbiology,
Nagoya City University Medical School,
Nagoya.

Hiroshi Ishihara

Chemical Laboratory,
Nagoya City University,
Nagoya, Japan.
Sept. 21.

[1] Linggood, F. V., and Wolwod, A. J., *Brit. J. Exp. Path.*, **30**, 93 (1949).
[2] Yoneda, M., *Jap. J. Bact.*, **5**, 401 (1950) (in Japanese).
[3] Yoneda, M., *Nature*, **167**, 860 (1951).
[4] Friedmann, T. E., and Haugen, G. E., *J. Biol. Chem.*, **147**, 415 (1958).
[5] Fowden, L., *Biochem. J.*, **48**, 327 (1951).
[6] Ishihara, H., and Yoneda, M., *Jap. J. Microbiol.*, **2**, No. 1 (1958) (in the press).

Thyroxine Metabolism by Extracts of Rat Liver

The *in vitro* de-iodination of thyroxine has been reported in a variety of biological systems, including slices, homogenates, and extracts of mammalian organs[1–3]. In particular, a system present in rat liver has been fairly extensively investigated and a technique evolved whereby quantitative measurements of de-iodination can be readily obtained[4]. At the outset of the present work, this technique was employed to re-investigate the properties of the de-iodinating system of rat liver. The heat stability of the homogenate[3] and the heat lability of the extract were confirmed, and the system shown to be inhibited by potassium cyanide. The rate of de-iodination was fastest at the beginning of the incubation period and fell off with time, levelling off after 1–2 hr. when 50–70 per cent de-iodination had occurred. Substrate specificity was not absolute, tri-iodothyronine being metabolized to a lesser extent. In all these respects the system used resembles that previously described by other workers[4].

All these preliminary experiments employed thyroxine labelled with iodine-131 in the 3′ : 5′ positions and chromatography of the incubation mixture failed to reveal any radioactive metabolites apart from iodide and a varying amount of excess thyroxine. Such chromatograms were frequently run together with a marker of stable tri-iodothyronine, but no trace of this compound could be identified among the labelled reaction products. It seems likely, therefore, that the two iodine atoms in the 3′ : 5′ positions are removed simultaneously, or very nearly so. It was thereupon decided to synthesize radioactive thyroxine labelled with iodine-131 in the 3 : 5 positions, and to trace the metabolic fate of these atoms in the same system. It was immediately apparent from the first trials that de-iodination from these positions was slight; 80–95 per cent of the radioactive label remained adhering to the protein of the extract, and was thus presumably still in organic combination, iodide passing through the filter. An experiment was also carried out in which the samples of thyroxine contained the label in varying proportions on the two rings. The results show a proportionality between the extent of de-iodination and the percentage of the label present in the 3′ : 5′ positions.

Percentage of label in 3′ : 5′ positions	De-iodination (per cent)
0	8·0
20	19·5
40	29·9
60	35·0
80	43·6
100	53·9

Thyroxine is thus apparently metabolized to a compound (or compounds) which contains the 3 : 5 iodine atoms but from which the 3′ : 5′ iodine atoms are absent. Work which is still in progress indicates that this unknown compound can be separated from excess thyroxine by butanol extraction. Its chemical structure is apparently such that the alanine side-chain and proximal ring are preserved intact, because, on treatment by hydrolytic procedures, a labelled degradation product is obtained having the chromatographic properties of 3 : 5 di-iodotyrosine.

This work was undertaken during tenure of a grant from the Rockefeller Trust of the Medical School, and with facilities granted by the Medical Research Council.

Lawrence G. Plaskett

Department of Clinical Research,
University College Hospital
Medical School,
London, W.C.1.

[1] Albright, E. C., Larson, F. C., and Tust, R. H., *Proc. Soc. Exp. Biol. and Med.*, **86**, 137 (1954).
[2] Larson, F. C., Tomita, K., and Albright, E. C., *Endocrinol.*, **57**, 338 (1955).
[3] Sprott, W. E., and Maclagan, N. F., *Biochem. J.*, **59**, 288 (1955).
[4] Maclagan, N. F., and Reid, D., CIBA Colloquia on Endocrinology, **10**, 190 (1957).

The Biuret Complex of Gelatin and the Mechanism of Gelation

The gelation of gelatin results from a thermally reversible cross-linking process involving hitherto unknown functional groups. In studies of gelation–molecular weight correlations of chemically modified gelatins (amino-acetylated, carboxyl-esterified, hydroxyl-acetylated, guanidino-nitrated) and of hypobromite-treated gelatin we have found that the polar and charged side-chain groups are of little importance in gelation[1]. Further, by study of the effects of

various electrolytes on the melting points of gels of gelatin and of chemically modified gelatins, we have found that binding of ions at the polar and charged side-chain groups is not the mechanism by which electrolytes affect gelation[2]. These results have directed our attention to the role of the peptide group.

By the use of the well-known biuret reaction, we now show that the peptide group plays an important part in gelation. Formation of the biuret complex of gelatin with as little as 0·03 *M* copper (II) or cobalt (II) chloride in 5 per cent gelatin (peptide group to metal ratio 17) at *p*H 10–12 caused reversible suppression of gelation. Such solutions did not gel at 0°. However, they did gel, almost instantaneously, at 0° on destruction of the complex and the characteristic violet-red colour ($\lambda_{max.}$ 550) by acetic acid, sodium cyanide or ion-exchange resin. The melting point of the decomplexed gel was 27°, while those of ordinary gels of the same concentration at *p*H 3–12 are 28–30°. Inhibition of gelation by alkaline copper is, therefore, caused by complex formation and not by the high *p*H or by hydrolysis. The gelation on destruction of the complex is a property of the gelatin, and is not caused by copper or cobalt ions, as was shown by independent experiments in which the metals were removed by ion-exchange or dialysis. Copper and cobalt in alkaline solution are the most effective melting-point reducers found so far. By comparison, sodium thiocyanate and lithium diiodosalicylate[3] in 0·03 *M* solution lower the melting point of the gel by 0·5° and 4°, respectively.

Copper complexes of gelatins having amino- or hydroxyl-groups quantitatively acetylated[3], carboxyl groups 85 per cent methylated[4] or guanidino-groups 70 per cent nitrated[1] also did not gel at 0°, but did gel after destruction of the complex by acidification. These results show that the inhibition of gelation is due to the copper–peptide complex and not to side-chain complexes.

There are several ways in which formation of the copper–peptide complex may prevent gelation. One is by blocking peptide groups that normally engage in cross-linking through hydrogen bonds. A second is by inhibiting a required configuration either by blocking the peptide groups which maintain the configuration through hydrogen bonding or by forming 'knots' that disrupt the configuration. The 'knots' may also prevent gelation by steric interference with intermolecular association. A combination of these causes may be operative.

Although inhibition of gelation of gelatin requires only one copper or cobalt ion per seventeen peptide groups, actually a larger portion of the peptide groups is complexed, since in biuret type complexes of $\lambda_{max.}$ 550 three or four peptide groups are linked to one metal atom. For example, the lowest polyglycine to give the complex is triglycylglycine, while dialanylalanine gives only a weak reaction[5]. Also, two copper atoms combine with the octapeptide leucylhexaglycylglycine[6], and one copper atom combines with two molecules of biuret[7]. Thus, the complexation of $\frac{1}{3}$–$\frac{1}{4}$ of the peptide groups appears adequate to prevent gelation, particularly as gelation of gelatin appears to involve not the random association of pairs of functional groups but of a number of groups in ordered segments or crystallites[8,9]. The maximum copper binding by gelatin has been reported as one to about six peptide groups[10], which we have confirmed. The ratio of four cannot be realized because of the proline and hydroxyproline residues which contain no replaceable peptide hydrogen. The fluid, rather than cross-linked, nature of the complex shows that the binding is intramolecular.

J. Bello*
J. R. Vinograd

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology,
Pasadena, California.
Oct. 31.

* Present address: Research Laboratories, Eastman Kodak Co., Rochester, New York.

[1] Bello, J., Riese, H. C. A., and Vinograd, J. R. (to be published).
[2] Bello, J., Riese, H. C. A., and Vinograd, J. R., *J. Phys. Chem.*, **60**, 1299 (1956).
[3] Bello, J., and Vinograd, J. R., *J. Amer. Chem. Soc.*, **78**, 1369 (1956).
[4] Bello, J., *Biochim. Biophys. Acta*, **20**, 426 (1956).
[5] Rising, M. M., Parker, F. M., and Gaston, D. R., *J. Amer. Chem. Soc.*, **56**, 1178 (1934).
[6] Plekhan, M. I., *J. Gen. Chem. U.S.S.R.*, **21**, 341 (1951).
[7] Schiff, H., *Ber.*, **29**, 298 (1896).
[8] Boedtker, H., and Doty, P., *J. Phys. Chem.*, **58**, 968 (1954).
[9] Eldridge, J. E., and Ferry, J. D., *J. Phys. Chem.*, **58**, 994 (1954).
[10] Nyilasi, J., and Kovats, Z., *Acta Chim. Acad. Sci. Hung.*, **2**, 451 (1952).

Autoradiographic Visualization of Synthesis of Deoxyribonucleic Acid in Tissue Culture with Tritium-labelled Thymidine

Thymidine is a specific precursor of deoxyribonucleic acid[1]. The very low energy of tritium electrons permits precise localization on autoradiographs (within less than one micron on average) of the atom that disintegrates. This is not possible with other radioisotopes[2] such as phosphorus-32 or even carbon-14 or sulphur-35.

Tritium-labelled thymidine was prepared by hydrogen exchange between ordinary thymidine (California Foundation for Biochemical Research) and tritiated water in the presence of freshly reduced Adams's catalyst at 100° C. for 24 hr. After the catalyst had been filtered off and the tritium water removed by distillation, the residue was heated in 0·2 *N* sodium hydroxide so that only firmly bound tritium was retained; the solution was then neutralized with sulphuric acid and evaporated to dryness. The labelled thymidine was extracted with hot butanol, and, after treatment with charcoal, crystallized twice from butanol. White needles melting at 185°–186° C. (corr.) were obtained which gave the ultra-violet spectrum of pure thymidine. The radioactivity determined in an ionization chamber after combustion of the compound and reduction of the water to hydrogen was 14·9 mc. per millimole; it was exclusively localized in the pyrimidine moiety, as it was found entirely in the thymine isolated after hydrolysis of the labelled thymidine. A complete description of this preparation has been published elsewhere[3].

To hanging-drop cultures of chick fibroblasts prepared on a specially thinned medium, tritium-labelled thymidine (2×10^{-3} *M*) in tyrode solution was added, either from the time of explantation or later, for various periods. After excision of the original explants (leaving only the growth zone on the coverslip) and careful washings in Tyrode solution to remove the tritium-labelled thymidine present outside the cells, the cultures were fixed in 80 per cent ethanol. The coverslips were then mounted upside down on slides and stripping-film autoradiographs were made.

Development of these autoradiographs showed very low background and the cytoplasms were never

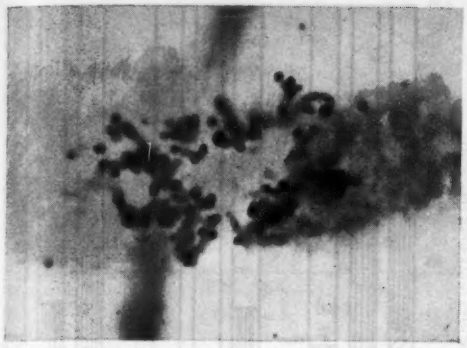


Fig. 1. Equatorial plate in a fibroblast. The chromosomes are grey. The sharp black dots are silver grains developed in the overlying autoradiographic film. × 2,100

labelled. Silver granules denoting the presence of tritium appear only over resting cell nuclei and on chromosomes in dividing cells (Fig. 1). The periphery of nucleoli often contains more grains than other areas of similar size in the nuclei. All labelled structures can be identified within less than 1μ, as already shown by Taylor, Woods and Hughes on another material[4]. The grains can be considered as indicative of the presence of tritium-labelled thymidine in an insoluble form, mainly incorporated into deoxyribonucleic acid.

When the culture medium has contained thymidine for 2 hr., a few nuclei, but no mitotic figures, are notably labelled. This number increases with time and, after long contact, all mitotic figures and most interkinetic nuclei are seen to contain tritium-labelled thymidine. But a variable number of nuclei (up to 30 per cent), mainly in the inner part of the growth zone, are free of silver grains even after three days of cultivation in the presence of the nucleoside. As they are intermingled with labelled cells, the possibility that they are too far from the film for the tritium electrons to reach it can be ruled out. It can thus be assumed that up to that number of cells do not participate in the mitotic cycle and that no turnover of deoxyribonucleic acid occurs in such cells. Auto-absorption, however, is an important factor, because there are more silver grains over large flattened nuclei than over small round ones. The ratio between nuclear area and number of grains can be analysed.

The ratio of labelled to the total number of mitotic figures has been determined after exposure to thymidine in the cultures for various lengths of time. The results are shown in Fig. 2. They make it clear that the stage of synthesis of deoxyribonucleic acid, involving nuclear incorporation of thymidine in insoluble compounds, occurs mainly between the sixth and eleventh hours before onset of mitosis in chick fibroblasts *in vitro* at 37° C. Independent evidence based on quantitative Feulgen determinations shows that synthesis of deoxyribonucleic acid precedes mitosis in the same material by 7–8 hr. on an average[5]; thus, there is near coincidence between fixation of this nucleoside and polymerization to deoxyribonucleic acid.

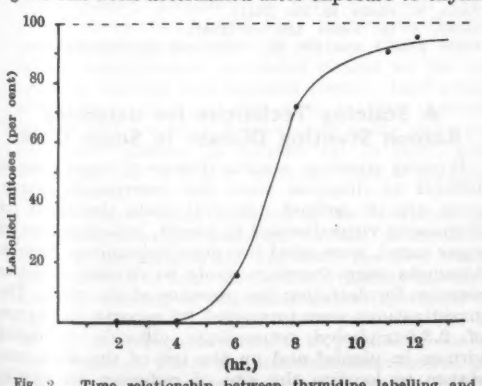


Fig. 2. Time relationship between thymidine labelling and mitosis

Counting the grains gives a fair estimate of the relative amount of thymidine present in structures of comparable thicknesses, such as the two chromosome groups of anaphases and telophases. The two always contain about the same number of grains. Deviation from equality in the two groups never exceeds what one would expect in a purely random process such as radioactivity. This is in accordance with the theory of equal division of deoxyribonucleic acid between the two daughter cells at mitosis, but not with the recent contrary findings of Plaut and Mazia[6] working on plant cells and using thymidine labelled with carbon-14 and consequently obtaining less accurate localization of the electron-emitting structure.

From these observations and others, to be published in detail elsewhere[5], it appears that tritium-labelled thymidine is a very useful tool for the cytological study of many aspects of the synthesis of deoxyribonucleic acid.

H. Firket
W. G. Verly

Institut d'Histologie
et Laboratoire des Isotopes,
Service de Pathologie générale,
Université de Liège.

[1] Reichard, P., and Estborn, B., *J. Biol. Chem.*, **188**, 839 (1951).
[2] Eidinoff, M. L., Fitzgerald, P. J., Simmel, E. B., and Knoll, J. E., *Proc. Soc. Exp. Biol. Med.*, **77**, 225 (1951).
[3] Verly, W. G., and Hunebelle, G., *Bull. Soc. Chim. Belg.*, **66**, 640 (1957).
[4] Taylor, J. H., Woods, P. S., and Hughes, W. L., *Proc. U.S. Nat. Acad. Sci.*, **43**, 122 (1957).
[5] Firket, H., *Arch. Biol.* (in the press).
[6] Plaut, W., and Mazia, D., *J. Biophys. and Biochem. Cytol.*, **2**, 573 (1956).

Reduction of Low-Temperature Injury to Stored Apples by Modulation of Environmental Conditions

Investigation of the metabolism of the senescent apple fruit has largely been directed to the practical objective of improving storage technique. Observations have generally been made on fruit kept in a constant environment. There is, however, no inherent reason why an environment favourable at one stage of post-harvest life should be equally favourable when applied at another stage. A. J. M. Smith[1] reported experiments that led to the adoption of a 'dual temperature' method in the refrigerated carriage of South African plums to the United Kingdom. I have already reported that with Victoria plums a change of temperature to 18° C. for two days on the seventeenth day after the commencement of storage at 0° C. resulted in a considerable delay in the subsequent appearance of the low-temperature injury described as 'internal browning'[2].

A similar result has now been obtained with the apple fruit (variety, Bramley's Seedling), although

the magnitude of the effect is less than with the plum. In Fig. 1 the amount of low-temperature injury, measured as the estimated percentage surface discoloured brown in 50 apples cut in half transversely to the stem-calyx axis, is plotted against a time-scale representing the period elapsing between the commencement of storage and the application of a warming treatment. The treatment consisted of raising the temperature of the fruit to 15° C. for 5 days after which it was returned to its original storage temperature of 0° C. All the apples were cut at the same date, 18 weeks after the commencement of storage. It will be seen that as the date of the treatment was advanced there was at first a progressive reduction in the extent and intensity of injury but that this was followed by a regular increase. The amount of injury when no warming treatment was given was comparable to that in samples which were warmed at the end of the experiment, five days before examination. The minimum injury occurred where warming was applied in the period 6–8 weeks after commencement of storage.

This picture was changed in a striking way when during the warming treatment the fruit was exposed to a current of carbon dioxide and air (Fig. 2). The amount of injury found after 18 weeks of storage was greatly reduced, the degree of reduction being greater when the concentration of carbon dioxide during warming was 20 per cent than when it was 10 per cent.

I have already suggested that the hypothesis put forward by Plank[3] that low-temperature injury may be due to the toxic effect of accumulating volatile metabolites is consistent with the effect of warming observed with plums[4].

Fidler[5] has shown that when carbon dioxide was allowed to accumulate to a concentration of 8 per cent during storage at 3° C. the output of total volatile substances from the apple was reduced. It would be interesting to see if temporary warming in an atmosphere containing carbon dioxide as described here can be shown to have an effect upon the production of total volatile substances. Work to this end is now proceeding.

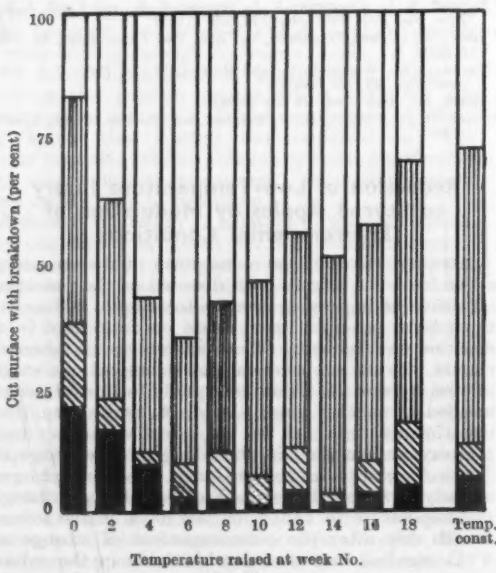


Fig. 1. Effect on occurrence of low-temperature breakdown of raising the temperature of apples (var. Bramley's Seedling) to 15° C. for five days at progressive intervals during storage at 0° C. (White, sound; vertical hatching, slight; oblique hatching, moderate; black, severe)

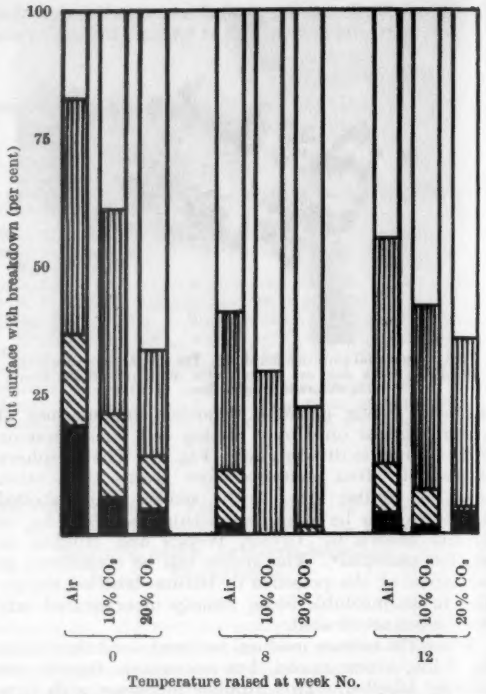


Fig. 2. Effect on occurrence of low-temperature breakdown of increasing the carbon dioxide content of the air in which apples (var. Bramley's Seedling) were kept for five days at 15° C. (White, sound; vertical hatching, slight; oblique hatching moderate; black, severe)

This paper was prepared as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

W. Hugh Smith

Ditton Laboratory,
Department of Scientific and
Industrial Research,
Larkfield, Maidstone.

[1] Smith, A. J. M., *J. Hort. Sci.*, **25**, 132 (1950).
[2] Smith, W. H., *J. Pomol. and Hort. Sci.*, **18**, 74 (1940).
[3] Plank, R., *Planta*, **32**, 364 (1941).
[4] Smith, W. H., *Nature*, **159**, 541 (1947).
[5] Fidler, J. C., *J. Hort. Sci.*, **25**, 2 (1950) and unpublished results.

A Staining Technique for detecting Ratoon Stunting Disease in Sugar Cane

Ratoon stunting, a virus disease of sugar cane, is difficult to diagnose since the macroscopic symptoms are ill defined. Several tests described for diagnosing virus diseases in plants, including one for sugar cane[1], were tried but gave inconsistent results. Attempts were therefore made to develop a colour reaction for detecting the presence of the virus. The investigations were prompted by reports on the use of 2,3,5-triphenyl tetrazolium chloride to detect viruses in plants[2] and on the use of the same substance to localize the site of reducing activity in sugar cane[3].

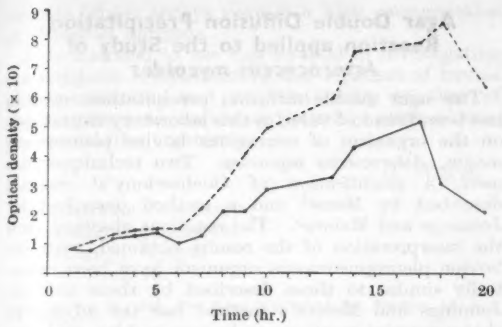


Fig. 1. Optical density of acetone extract. Full line, healthy; broken line, diseased

The test is based on the reduction of the soluble, colourless tetrazolium salt into the insoluble red formazan in the presence of viable tissues. It should be pointed out that little is known about the reducing mechanisms in the tetrazolium reaction. Furthermore, attention has been directed[4,5] to the photochemical properties of tetrazolium, a factor which demands carefully controlled conditions in experimentation.

The experimental procedure was as follows. Samples of cane tissue were taken from nodes of sugar-cane stalks by means of a cork borer cutting a plug 1 cm. in diameter. After sectioning the stalk at the level of the leaf scar, the plug was removed by boring vertically down into the lower internode, just inside the rind and opposite the bud. Slices, 0·5 mm. thick, were prepared by means of a hand microtome and the six sections immediately above the level of the leaf-trace bundles were selected. These were placed in 0·5 per cent solution of 2,3,5-triphenyl tetrazolium chloride and kept in an incubator, in darkness, at 35° C. The insoluble red formazan was extracted twice from the sections using each time 5 ml. of hot acetone. The optical density of the solution was determined by means of a Lumetron colorimeter, standardized with distilled water.

The change in colour was found to be more rapid with virus-infected than with corresponding virus-free tissue. The basal nodes of mature sugar cane stalks gave the best difference in colour intensity between healthy and diseased cane. Maximum difference was obtained when the four consecutive nodes above ground-level were sampled. When young canes were tested they reacted in the same way as young portions of mature cane, that is, colour development proceeded almost at the same speed in healthy and diseased tissue. Leaf punches taken from the mid-ribs also proved unsuitable for the test. Colour development being more rapid when the reaction is carried out in light, colour differentiation is better when the test is performed in darkness.

Cane tissue, when sampled in the internode, gave a minimum to no reaction. When sections were taken at the level of the leaf-trace bundles, reaction was too rapid in both infected and healthy tissue, and the large number of vascular bundles interfered with the development of a good colour pattern. The best zone for sampling was found to be just above the leaf-trace bundles, where the intermediate reaction gave a maximum difference in change in colour between healthy and diseased tissue.

The following sampling procedure was finally adopted. Six slices were obtained above the level of the leaf-trace bundles of the second and third nodes above ground-level. The twelve sections obtained were tested together for each stalk. The reaction was allowed to proceed for different lengths of time and the extraction carried out at intervals. The results are expressed graphically in Fig. 1. Although a difference in colour can be observed after 2 hr. when the reaction takes place in darkness, the best difference is obtained after, approximately, 9 hr. and is still very marked after 20 hr. It will be noted that there is a drop in both curves after seventeen to eighteen hours. It has been observed that the red colour fades and eventually disappears in the slices when left in the reagent, as well as in the acetone extract. It is therefore important to carry out the test under carefully controlled conditions.

More than 1,000 nodes were sampled from presumed healthy and diseased *M.*134/32 and *D.*109 canes growing in ratoon stunting field trials. Disease assessment by means of the colour test and macroscopic nodal symptoms[6] were in close agreement.

The method has its limitations in that it is applicable only to mature cane, and several varieties still have to be tested. The results obtained can be of only preliminary nature, and a more exhaustive study is being conducted.

Other tetrazolium salts, namely 3,3-dianisole-*bis*-4,4-(3,5-diphenyl)-tetrazolium chloride and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride, were tested but did not show promise as colour indicators.

ROBERT ANTOINE

Sugar Industry Research Institute,
Réduit, Mauritius.

[1] Farrar, L. L., *Phytopathology*, **47**, 10 (1957).
[2] Beal, J. M., Preston, jun., W. H., and Mitchell, J. W., *Plant Dis. Rept.*, **39**, 558 (1955).
[3] Dufrenoy, J., and Pratt, R., *Amer. J. Bot.*, **35**, 333 (1948).
[4] Jambor, B., *Nature*, **173**, 774 (1954).
[5] Jambor, B., *Nature*, **176**, 603 (1955).
[6] Hughes, C. G., and Steindl, D. R. L., Tech. Com. No. 2 Bureau of Sugar Experiment Stations, Queensland (1955).

Occurrence of a Blue-Green Alga in the Nodules of *Trifolium alexandrinum*

DURING a cytochemical study of nodule cells in Egyptian clover, *Trifolium alexandrinum*, it was observed that in addition to the rhizobial bacteria, a blue-green alga, identified as *Nostoc punctiforme*, occurred inside the nodules. So far as we are aware, blue-green algae have not been reported to be associated with nodule formation in legumes.

The alga, at first, occupies the intercellular spaces in the nodules (Fig. 1, 2) and gradually invades the cellular region, forming globose to subglobose colonies (Fig. 1, 1). The trichomes are few-celled, though many occur as individual cells. The characteristic blue-green pigment, double-walled akinetes, intercalary heterocysts and yellowish-brown coloration with iodine help to distinguish the alga from the associated bacteria (Fig. 1, 3). The alga could be successfully cultured in sterilized Erdschreiber soil solution. The alga occurs only in the nodules of *T. alexandrinum*, the other parts of the plant showing no such organism.

Nodules of both diploid and tetraploid *T. alexandrinum* showed the alga, as well as those of *T. alexandrinum* collected from different localities. The alga was, however, absent in the nodules of *T.*

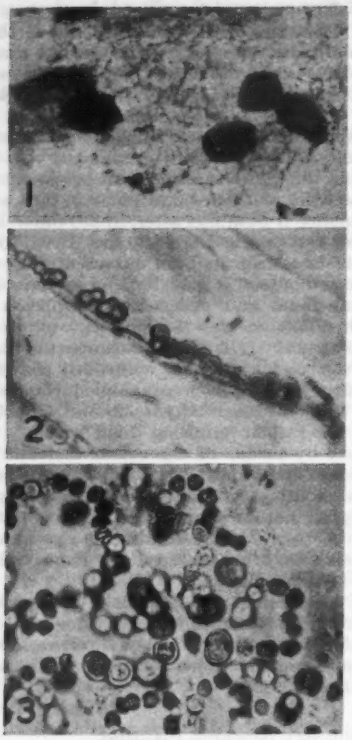


Fig. 1. (1) Colony of *Nostoc punctiforme* inside the root nodule. (2) Individual cells of the alga in the intercellular space inside the nodule. (3) *Nostoc punctiforme*, from culture, showing akinetes and heterocyst

pratense, *T. hybridum*, *Lupinus alba* and *Melilotus alba*, which were growing in the same fields as *T. alexandrinum* or in adjoining areas.

In some lower plants, blue-green algae, particularly those belonging to the genera *Nostoc* and *Anabaena*, have been reported to occur endophytically[1]. The occurrence of *N. punctiforme* in root nodules alone suggests that the relationship may not be a simple case of endophytism. Many blue-green algae are known to fix atmospheric nitrogen[2–4], and it is possible that *N. punctiforme* plays some such part. The reasons for the specific affinity of the alga to *T. alexandrinum* are also not clear. This legume is widely cultivated as a winter fodder in north India under irrigated conditions, and we are now studying the precise relationship between the alga and the leguminous nodule as well as the nitrogen-fixing ability of the alga.

We are indebted to Dr. M. S. Randhawa, Dr. B. P. Pal and Dr. S. M. Sikka for their interest in the study and encouragement. We are also grateful to Dr. M. S. Swaminathan for advice.

S. Bhaskaran
G. S. Venkataraman

Division of Botany,
Indian Agricultural Research Institute,
New Delhi, 12.

[1] Fritsch, F. E., "Structure and Reproduction of Algae", **2**, 872 (Cambridge, 1935).
[2] Fogg, G. E., *J. Exp. Biol.*, **19**, 78 (1942).
[3] De, P. K., *Proc. Roy. Soc.*, B, **127**, 121 (1939).
[4] Singh, R. N., *Indian J. Agric. Sci.*, **12**, 743 (1942).

Agar Double Diffusion Precipitation Reaction applied to the Study of *Asterococcus mycoides*

The agar double diffusion precipitation reaction has been found of value in this laboratory in research on the organism of contagious bovine pleuropneumonia, *Asterococcus mycoides*. Two techniques are used, a modification of Ouchterlony's[1] method described by Mansi[2] and a method described by Jennings and Malone[3]. The reactions observed, and the interpretation of the results obtained, with the bovine pleuropneumonia organism have been essentially similar to those described by these authors. Jennings and Malone's method has the advantage that a positive reaction can be seen in 2 hr., and that more distinct separation of lines of precipitation occurs as diffusion proceeds; but larger quantities of serum and antigen are required than for the method of Mansi.

Immune sera are produced in rabbits by intravenous injections of increasing doses of washed organisms grown in a culture medium containing no bovine antigens[4]. No reaction is produced between normal rabbit serum and infected bovine tissues or extracts of the organism, or between immune rabbit serum and normal bovine tissues. When immune serum is tested against material from infected tissues, such as pleural exudate or a saline extract of a lung lesion, a strong reaction is seen. Usually three lines of precipitation are apparent, demonstrating that the organism contains at least three soluble antigens. Cultures and extracts of the organism also give the same result. All strains of the organism studied here have produced the three lines, indicating no qualitative difference in the antigenic structure of different strains.

Most areas in tropical Africa where contagious bovine pleuropneumonia is enzootic are relatively isolated, and communications with laboratories are therefore often slow and unsatisfactory. In these circumstances a laboratory diagnosis on specimens obtained post-mortem is usually difficult or impossible through lack of suitable fresh material from the field, and reliance must be placed on serological tests of other animals in the affected herd. Consequently a technique which enables a suspected diagnosis of pleuropneumonia to be confirmed at the laboratory by the examination of preserved, or of partially decomposed and contaminated, material would be most useful. In order to investigate the value of the precipitation reaction in these conditions, two samples of unpreserved contaminated material from bovine lung lesions were kept at room temperature and tested at intervals. The reaction remained positive for up to 13 days, by which time the specimens were completely putrified. Cultural examination of the two samples in a suitable medium, containing thallium acetate and penicillin to suppress other bacteria, failed on and after the second and third days respectively, due to gross contamination. It has been found that 5 per cent formalin and 50 per cent glycerin do not destroy the antigen, and the test can be satisfactorily carried out on infected tissues preserved with these reagents.

The sera of cattle dying of infection with *Asterococcus mycoides*, when tested against immune rabbit sera, often give one or two lines of precipitation, demonstrating the presence of circulating antigens, probably absorbed from the extensive lesions and

exudate which always contain a high concentration of diffusible antigens.

It is intended to use this method in investigating the antigenic composition of the organism of bovine pleuropneumonia, and its relationship to that of caprine pleuropneumonia and possibly other pleuropneumonia-like organisms.

G. WHITE

East African Veterinary
Research Organization,
Muguga, Kenya.
Oct. 10.

[1] Ouchterlony, O., *Ark. For. Kemi Mineralogi. Oct. Geologi.*, **26**, B, 1 (1948).
[2] Mansi, W., *J. Comp. Path.*, **67**, 297 (1957).
[3] Jennings, R. K., and Malone, F., *J. Immunol.*, **72**, 411 (1954).
[4] Newing, C. R., and MacLeod, A. K., *Nature*, **177**, 939 (1956).

Incompatibility in *Theobroma cacao*

Knight and Rogers[1] postulated the existence of five incompatibility alleles, at a single locus, in the three self-incompatible but cross-compatible cacao genotypes studied by them. The five alleles differed in dominance, but two were equal; the same dominance sequence, $S_1 > S_2 = S_3 > S_4 > S_5$, was exhibited in both male and female parts of the flower. Their criterion of compatibility and incompatibility was the retention or fall, respectively, of hand-pollinated flowers guarded from insect visits.

I have used a different approach to the problem. Microtomed sections of pollinated ovaries have been microscopically examined. In all cases of self- and cross-incompatibility, a proportion of fertilized ovules shows non-fusion between the egg nucleus and first male gamete and between the polar nuclei and second male gamete; the remaining ovules show normal fusion. Three main types of self-incompatible tree are now recognized wherein the proportion of 'non-fusion' ovules is: (1) 25 per cent; (2) 50 per cent; (3) close to or actually 100 per cent. Compatible pollinations, on the other hand, give all normal ovules. *Theobroma cacao* L. provides, for the first time, an example of an incompatibility mechanism in the flowering plants which has non-fusion of gametes as its fundamental manifestation, the ratios of ovules wherein non-fusion and fusion of gametes take place bearing simple genetic ratios one to the other (1 : 3, 1 : 1, 1 : 0).

On a visit to the West African Cacao Research Institute in 1956, I was privileged to use some of the trees investigated by Knight and Rogers. Pollinated ovaries from guarded flowers were fixed, sectioned and examined.

All genotypes representing a combination of an allele and another recessive to it (for example, $S_{1\cdot2}$, $S_{2\cdot5}$, $S_{3\cdot4}$) gave, on self-pollination, counts wherein the proportion of non-fusion ovules was 25 per cent. The $S_{2\cdot3}$ genotype, a combination of alleles of equal dominance, gave 50 per cent non-fusion ovules on self-pollination. The $S_{5\cdot5}$ genotype did not, however, give on selfing the expected 100 per cent non-fusion content; instead, it gave good 25 per cent counts, suggesting that, in fact, it is not homozygous, but is a combination of S_5 with a second allele recessive to it (say, S_6). The consequences of this assumption do not in any way invalidate the findings of Knight and Rogers.

Table 1. PERCENTAGE RATIOS OF 'NON-FUSION' TO NORMAL OVULES IN CACAO SELFINGS AND CROSSES. INCOMPATIBLE COMBINATIONS IN BOLD-FACE TYPE

Female	Male				
	$S_{1\cdot2}$	$S_{2\cdot3}$	$S_{2\cdot5}$	$S_{3\cdot4}$	$S_{5\cdot5}$
$S_{1\cdot2}$	**25**	0	0	0	0
$S_{2\cdot3}$	0	**50**	**25**	**25**	0
$S_{2\cdot5}$	0	**25**	**25**	0	0
$S_{3\cdot4}$	0	**25**	0	**25**	0
$S_{5\cdot5}$	0	0	0	0	**25**

Cross-pollinations between genotypes carrying the same dominant allele (for example, $S_{2\cdot3} \times S_{2\cdot5}$) also gave 25 per cent non-fusion ovules in the ovary. The cross, $S_{2\cdot3} \times S_{3\cdot4}$, found by Knight and Rogers to be incompatible, likewise gave 25 per cent non-fusion ovules.

All combinations that do not involve the same dominant allele (for example, $S_{1\cdot2} \times S_{2\cdot5}$, $S_{1\cdot2} \times S_{3\cdot4}$) and those that do not involve a duplication of alleles of equal dominance in the presence of the other (for example, $S_{2\cdot5} \times S_{3\cdot4}$) were found to give only normal ovules in the pollinated ovaries. Knight and Rogers found these combinations to be compatible.

The results are summarized in Table 1, which shows the percentage proportion of non-fusion ovules counted. The principal conclusions to be drawn from the results are: (1) that gametes carrying the same dominant allele are those that do not fuse in the embryo-sac; (2) that the fusion or non-fusion behaviour of gametes is determined by the S allele they carry interacting with the diploid tissues (for example, S_2 gametes fuse in $S_{1\cdot2}$ tissues, but not in $S_{2\cdot5}$ tissues), so that incompatibility or compatibility is ultimately determined by the diploid genotype and hence is sporophytically controlled; (3) the similarity of reciprocal pollinations.

From my observations on the incidence of compatibility and incompatibility in progenies arising from crosses between self-compatible and self-incompatible parents, the hypothesis put forward by Knight and Rogers, based on the operation of an allelic series at a single locus, appears inadequate. It seems necessary to postulate the existence of two independent loci, one (P,p) concerned with the production of an 'incompatibility precursor', and another (S), which imparts specificity to the precursor. The P,p locus shows simple dominance and recessivity, and acts before meiosis; the S locus shows allelomorphism and acts after meiosis. If either locus is inactive (pp and $S_{f\cdot f}$, where the f indicates the presence of an amorph), full self-compatibility results. There will therefore be three main types of self-compatible tree: (1) $pp\ S_{a\cdot b}$ (self-compatible by virtue of the non-production of the precursor); (2) $PP\ S_{f\cdot f}$ (self-compatible by virtue of amorphism at the S locus); (3) $pp\ S_{f\cdot f}$ (both loci inoperative). A cross between the first, believed to be representative of the Central-American self-compatible tree, and the second, thought to represent the Trinidad self-compatible, will produce offspring with both loci active, and hence all will be self-incompatible. This result has been obtained.

F. W. COPE

Regional Research Centre,
Imperial College of
Tropical Agriculture,
Trinidad.

[1] Knight, R., and Rogers, H. H., *Nature*, **172**, 164 (1953); *Heredity*, **9**, 69 (1955).

A Chromatographic Method for studying Simple Compounds in Plants

Most chromatographic methods of investigating simple compounds (for example, saccharides and amino-acids) and their distribution in plant tissues have involved preparing extracts either of the whole plant or of dissected portions.

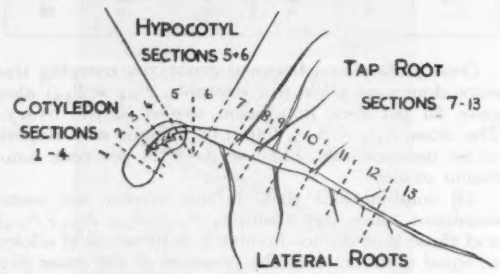


Fig. 1. Sectioned bean

I would like to indicate the possibilities of a less-elaborate technique to attain similar or more defined results. The method, comparable in principle to that of La Cour and Drew[1], is essentially to press the sap juices of the plant in question directly on to the chromatogram; instead of preparing water or alcohol extracts, concentrating and placing these on the chromatogram.

The plant studied in the initial experiments was a 14-days old seedling of the French bean *Phaseolus vulgaris*, var. Improved Canadian Wonder. This plant provided large amounts of sap, but other experiments with more woody plants also gave satisfactory results.

The beans were germinated for 14 days and then used immediately or stored at −20° C. until required.

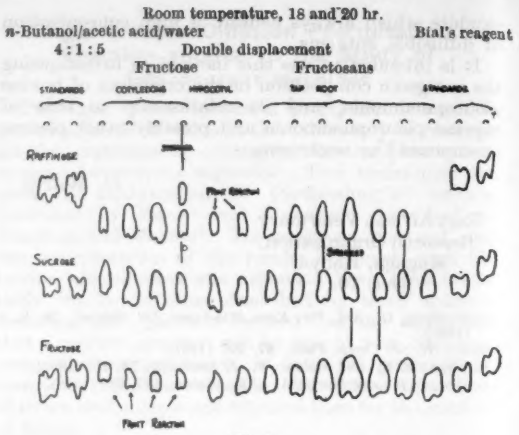


Fig. 3

(Stored beans were allowed to regain room temperature slowly before use.) The testa of the bean was removed and the plant divided into sections as shown in Fig. 1.

Each section was then placed at a marked position on the starting line of a paper chromatogram (Whatman No. 4, 22 in. × 18 in.), and its sap expressed by hand pressure exerted on a glass plate (4 in. × 6 in.) on top of the section. Underneath the chromatogram was another glass plate to prevent liquid loss and damage to the paper. When the sap had been absorbed by the paper and had spread to the desired spot size (¼ in. diam.), it was rapidly dried in a stream of hot air (30 sec.–1 min.). The process was repeated until most of the sap was removed from the section leaving only the skeleton of the section, which was discarded.

Descending chromatography was then carried out using conventional techniques for the compounds under investigation.

By use of the appropriate series of sections, the sap components of a whole bean could be assembled on the paper in such a way as to indicate their distribution in the bean (Fig. 2). No detailed report on the findings will be attempted here; but, in summary, the results of the initial experiments showed that: (*a*) the method was applicable to such compounds as saccharides, including fructose and fructosans (Figs. 2 and 3), amino-acids and ultra-violet-absorbing substances; (*b*) there was an unequal distribution of these simple compounds between the cotyledons and the tap root. For example, fructose was found in the tap root but little was found in the cotyledons (Fig. 3). Proline was found in the cotyledons but not in the tap root, where a ninhydrin-reacting compound appeared to run to the same position; (*c*) the hypocotyl contained the smallest number of compounds.

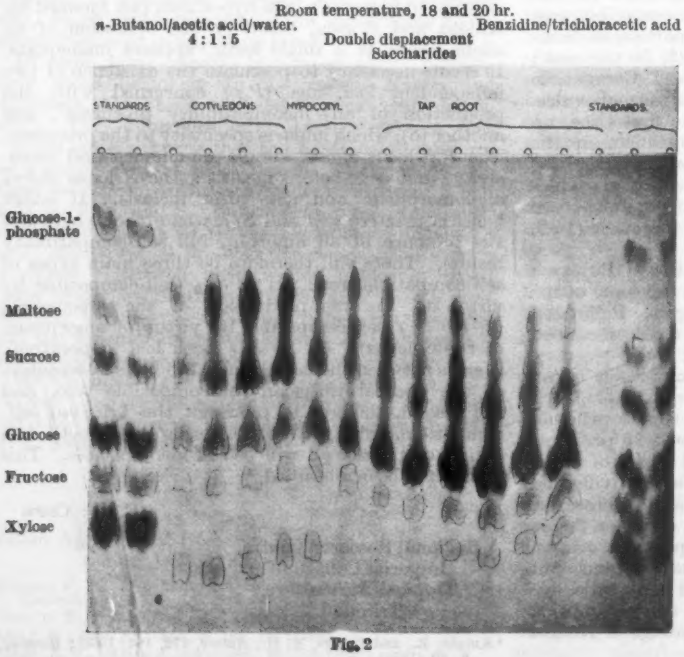


Fig. 2

This method may thus be of use in studying the distribution of simple compounds in the sap of whole or portioned plants during germination, and its application may also provide some information on translocation and other allied problems.

An objection to this method could be that the enzymes released by crushing are active in the time it takes to dry the sap and at the elevated temperatures of drying: this would complicate the picture observed. However, the sap is dried in a very short time, especially if an efficient hot-air dryer is used (30 sec. for the root section and 1–1½ min. for the cotyledon sections), thus minimizing enzyme action. In later experiments the area on which the sap was to be placed was impregnated with 5 μl. of a 0·01 *M* mercuric chloride solution[2] as an enzyme inhibitor and results were similar to those with spots submitted solely to heat treatment. It seems evident that enzyme action is so slight as to be negligible.

R. N. Greenshields

Department of Applied Biochemistry,
University of Birmingham.
Edgbaston, 15.

[1] La Cour, L. F., and Drew, R., *Nature*, **159**, 307 (1947).
[2] Bealing, F. J., and Bacon, J. S. D., *Biochem. J.*, **53**, 277 (1953).

Fluorescent Substances produced by Dermatophytes

The fluorescent substances formed by *Microsporum* species and *Trichophyton schoenleini* when attacking hair *in vivo* have not been identified; recently Wolf[1], on the basis of *in vitro* studies of the growth of *M. canis* and *M. gypseum* on hair, has produced evidence indicating that one of the substances is a pteridine. It is felt that this conclusion should be accepted with reserve for the following reasons.

(1) Wolf has produced no evidence to eliminate the possibility that the fluorescent products obtained could not also have been prepared from uninfected hair; Chattaway and Barlow[2] have shown that fluorescent materials can be extracted by water from normal hair, but that these have different chromatographic and electrophoretic properties from those formed during fungal attack *in vivo*.

(2) No comparison was made with fluorescent materials extracted from naturally infected hair. It is well known (for example, Vanbreuseghem[3]) that the mode of attack of hair *in vitro* by these fungi differs markedly from that seen *in vivo*. The metabolism of the organism is likely, therefore, also to differ, and any fluorescent products obtained during *in vitro* growth cannot be assumed to be identical with those formed *in vivo* unless direct comparison of properties is made.

(3) Chattaway and Barlow showed that aqueous extraction of hair damaged by fungi removed a complex mixture of substances, and treatment of such an extract with charcoal and methanol as described by Wolf would almost certainly not effect any great purification of the fluorescent material. Since the evidence for the material being a pteridine rests on absorption spectra data, any correlation of such data with fluorescent substances is at least very uncertain without further purification of the materials.

(4) Chemical examination by Davidson and Gregory[4] of aqueous extracts from naturally infected hair showed the presence of nitrogen, an aldehyde group and a phenolic ring; while Robinson, Figge and Bereston[5] concluded from infra-red analyses that the fluorescent material was a peptide. Chattaway and Barlow confirmed the findings of Davidson and Gregory but showed that the phenolic substance could be separated from the fluorescent substances by chromatography and that the fluorescence was quenched by bromine water. It is difficult to interpret these findings as supporting the presence of a pteridine.

F. W. Chattaway

Department of Biochemistry,
University of Leeds.

A. J. E. Barlow

Department of Dermatology,
The Royal Infirmary,
Huddersfield, Yorks.

[1] Wolf, F. T., *Nature*, **180**, 860 (1957).
[2] Chattaway, F. W., and Barlow, A. J. E., *J. Gen. Microbiol.*, **11**, 506 (1954).
[3] Vanbreuseghem, R., *Ann. parasitol.*, **24**, 559 (1949).
[4] Davidson, A. M., and Gregory, P. H., *Canad. J. Res.*, **7**, 378 (1932).
[5] Robinson, jun., H. M., Figge, F. H. J., and Bereston, E. S., *Amer. Med. Assoc. Arch. Dermatol. Syph.*, **68**, 129 (1953).

Streptomycin and Bacterial Respiration

There are many reports in the literature of effects of streptomycin on bacterial respiration[1]. Most of these involved the addition of streptomycin to non-growing suspensions of bacteria; but it seems likely that changes occurring during growth in the presence of streptomycin might ultimately prove of greater significance.

In the experiments described here, the effects on respiration of *Staphylococcus aureus* produced by exposure to dihydrostreptomycin during growth have been investigated.

Each experiment was performed as follows. Meat digest broth (50 ml. in a 250-ml. Erlenmeyer flask) was inoculated with *Staph. aureus* from an 18-hr. shaken culture in the same medium, to give a just-visible opacity, and the inoculated medium was incubated with continuous shaking for 2 hr. The density was then estimated absorptiometrically, and the culture was divided into two 25-ml. portions in 250-ml. flasks. The cultures at this stage contained staphylococci equivalent to about 1 mgm. dry wt. per ml. To one of these, dihydrostreptomycin in the required concentration was added (3 μgm.–50 μgm./ml.), and incubation of the flasks was continued, with shaking, for a further 2 hr. The cells were then harvested and washed three times with distilled water, by centrifugation. Suspensions were made in 0·1 *M* phosphate buffer, *p*H 7·3, and oxygen uptake with and without added substrate was measured for 30 min. by the conventional Warburg technique, no further dihydrostreptomycin being added at this stage. The dry weight of cells in the manometric experiments was 5 mgm./flask.

With succinate as substrate, the rate of oxygen uptake by cells which had been exposed to dihydrostreptomycin during growth was strikingly lower than that of the control cells. The decrease of oxygen uptake with glucose was, however, less marked and did not vary much with alteration of concentration of dihydrostreptomycin during growth. All figures are corrected for endogenous oxygen uptake of the respective cells, and are given to nearest 5 per cent.

Further experiments have shown that, under similar conditions, rate of uptake of oxygen with pyruvate is less affected than rate with succinate. For example, after exposure to 25 μgm. dihydro-

Table 1

No. of exp.	Oxygen uptake, dihydrostreptomycin-exposed cells with succinate (Control = 100)	Oxygen uptake, dihydrostreptomycin-exposed cells with glucose (Control = 100)	Di-hydrostreptomycin concentration during growth. (μgm./ml.)	Total growth of dihydrostreptomycin-exposed cultures on dry-weight basis (Control = 100)
1	10	80	50	35
2	15	85	25	35
3	30	85	12	65
4	40	80	6	85
5	60	80	3	90

streptomycin/ml. during growth, rate of oxygen uptake with succinate was decreased by 85 per cent whereas with pyruvate the decrease was 20 per cent.

The effects described have not been called 'inhibition' of oxygen uptake, since it is possible that the underlying mechanism might be an altered balance of enzymes in the cells, due to differential effects of streptomycin on enzyme synthesis, and not a direct interference with oxidative enzymes already present. If the organisms are harvested less than 2 hr. after addition of dihydrostreptomycin, lesser degrees of reduction of oxygen uptake are found. With 25 μgm. dihydrostreptomycin/ml. present during growth, for example, the growth-curves of the dihydrostreptomycin and control cultures differ only slightly in the first ½ hr. of incubation, but thereafter diverge rapidly. The effects on oxygen uptake with succinate follow roughly the same pattern.

It is interesting to note that the streptomycin antagonist (2-heptyl-4-hydroxyquinoline-N-oxide) investigated by Lightbown and Jackson[2] strongly inhibits the oxidation of succinate if added to non-growing suspensions of *Staph. aureus*, whereas dihydrostreptomycin itself produces no measurable inhibition with this organism under these conditions.

Thanks are due to Mr. R. W. Warner for technical assistance.

F. L. Jackson
(Cowburn Research Fellow)
King's College Hospital Medical School,
Denmark Hill,
London, S.E.5.

[1] Oginsky, E. L., *Bact. Rev.*, **17**, 17 (1953).
[2] Lightbown, J. W., and Jackson, F. L., *Biochem. J.*, **63**, 130 (1956).

Inhibition of Blood Digestion in Mosquitoes by Cations, and Cation–Antibiotic Mixtures

During the course of some studies originally designed to evaluate the role of certain cations and anions in influencing the susceptibility of *Aedes aegypti* to infection with *Plasmodium gallinaceum*, it became apparent that the calcium fed to these mosquitoes was also consistently inhibiting digestion of the ingested blood meal in a fairly constant proportion of the mosquitoes. This report extends this observation somewhat, and deals with some preliminary experiments on the inhibitory effects of the cations calcium and magnesium, alone and in combination with the antibiotic oxytetracycline, on the process of blood digestion in the mosquito *A. aegypti*.

In these experiments, the salts, antibiotic, and salt and antibiotic mixtures were added in the appropriate concentrations to a 4 per cent sugar nutrient solution which is generally used in this laboratory as the maintenance diet for mosquitoes. The solutions were made available to the adult mosquitoes immediately after pupal emergence was completed, and

Table 1. The Effect of Feeding Cations, and Cation and Antibiotic Mixtures, on Blood Digestion in *Aedes aegypti*

Regimen	Mosquitoes containing blood residues	Total mosquitoes	Mosquitoes containing residues (per cent)
Sugar	0	160	0·0
0·002 *M* oxytetracycline	0	172	0·0
0·1 *M* calcium chloride	52	166	31·3
0·1 *M* calcium chloride + 0·002 *M* oxytetracycline	117	164	71·3
0·1 *M* magnesium chloride	2	162	1·2
0·1 *M* magnesium chloride + 0·002 *M* oxytetracycline	64	165	38·7

the mosquitoes were then maintained on these solutions for the following 72 hr. At the end of this period the mosquitoes were allowed to gorge on normal chicks. Following the blood meal, the mosquitoes were allowed to continue to feed on the drug solutions until the experiments were terminated. Normally, in mosquitoes maintained at a constant laboratory temperature of 26·5° C. and a constant relative humidity of 75 per cent, as in this case, a blood meal will have been completely digested within 72 hr. At the end of this period the stomach will be entirely empty and contain no microscopically discernible trace of the blood meal. In these experiments, in order to emphasize the extent of inhibition of blood digestion, dissection of the mosquito and examination of the stomach was delayed until the fourth day following the blood meal. In those mosquitoes in which digestion has been inhibited the blood remains as a kind of darkened, amorphous mass, about one-third the size of the clot resulting from the original blood meal. Microscopically, intact red cells are no longer evident although the residue still retains the characteristic orange-reddish colour of hæmoglobin. Chemically, the residue gives a positive test for hæm by the benzidine reaction.

It is evident from the data presented in Table 1, which summarizes the results of several experiments, that calcium markedly inhibited digestion, since 31·3 per cent of the blood-fed mosquitoes still contained an undigested residue 96 hr. after the original feeding. At the same time, oxytetracycline alone had no apparent inhibitory effect, since none of the mosquitoes fed on that compound contained any discernible blood residues even when they were examined as early as 72 hr. after the blood meal. When it was fed to the mosquitoes in combination with calcium, however, oxytetracycline markedly increased the inhibitory effects of calcium and as a result the proportion of mosquitoes still containing considerable blood residues rose to 71·3 per cent of the total animals in the group. This potentiating effect of oxytetracycline was even more evident when it was fed in combination with magnesium. Thus, as shown in Table 1, magnesium alone had almost no inhibitory effect, but when it was fed together with oxytetracycline, 38·7 per cent of the mosquitoes contained undigested residues 96 hr. after the blood meal. It may be noted here that chloride, fed to the mosquitoes in 0·02 *M* concentrations of hydrochloric acid, either alone or with oxytetracycline, had no discernible effect.

It is of interest to note that when 0·058 *M* concentrations of the trisodium salt of ethylenediamine tetraacetic acid are added to the solutions of calcium and magnesium alone, or to the solutions of calcium and magnesium combined with oxytetracycline, so that these ions are in effect removed by the resulting

chelation, their inhibitory effects are simultaneously eliminated and blood digestion proceeds normally. Thus, this fact lends further support to the assumption that these inhibitory effects are produced primarily by the cations, and that the antibiotic acts rather by potentiating and activating either the cations or the reaction between the cation and the particular enzyme system involved, in a manner not yet understood, but differing from the usual reaction between cation and antibiotic[1] in which the activity of the one or the other is either eliminated or inhibited. It has been observed, too, that those mosquitoes containing large, undigested residues nevertheless produce eggs in great numbers. Even though egg-laying is apparently delayed in such mosquitoes, as evidenced by the fact that unlike normal eggs these eggs become heavily pigmented while still in the body, the fact that eggs are produced in quantity indicates that there has been no interference with digestion or assimilation of the plasma fraction of the ingested blood meal, since it is well known that it is this fraction which the mosquito requires for egg laying. In view of this, and other data presented above, it appears reasonable to assume that only digestion of the hæmoglobin fraction, of all the fractions contained in the original blood meal, is affected by the cations, or cation and antibiotic mixtures.

LEVON A. TERZIAN

Naval Medical Research Institute,
National Naval Medical Center,
Bethesda, Maryland.

[1] Weinberg, E. D., *Bact. Rev.*, **21**, 46 (1957).

Response of the Adrenal Medulla of the Sheep Fœtus to Asphyxia

OCCLUSION or ligation of the umbilical cord of the fœtal lamb is followed by a rise in arterial blood pressure and changes in heart-rate[1–5]. These reactions, which have been attributed to asphyxia, can be first elicited after 80–90 days of pregnancy and become more pronounced in the mature fœtus. The magnitude of the reactions has been related to the development and maturation of the autonomic nervous system[5].

In the present experiments the output of adrenaline and noradrenaline from the adrenal gland has been studied in fœtal lambs of different ages from 80 days of pregnancy until about term (150 ± days). The fœtus was removed from the uterus under pentobarbitone sodium anæsthesia, but the umbilical cord and the placental circulation were retained until the induction of asphyxia. Venous effluent blood was collected from the left adrenal gland after heparin (1,000 I.U./kgm.) had been injected into both the maternal and fœtal circulations. The left splanchnic nerve was cut and, when necessary, the peripheral end stimulated from an induction coil. Asphyxia was induced by ligation of the umbilical cord; a mask placed over the mouth of the fœtus prevented distension of the lungs or respiratory exchange. The adrenaline and noradrenaline content of the blood and of the adrenal glands was estimated by the method of Vogt[6].

The resting level of adrenaline and noradrenaline secretion from the denervated adrenal medulla is normally very low[6]; this was confirmed for the fœtal lamb. After ligation of the umbilical cord, large amounts of the sympathomimetic amines appeared

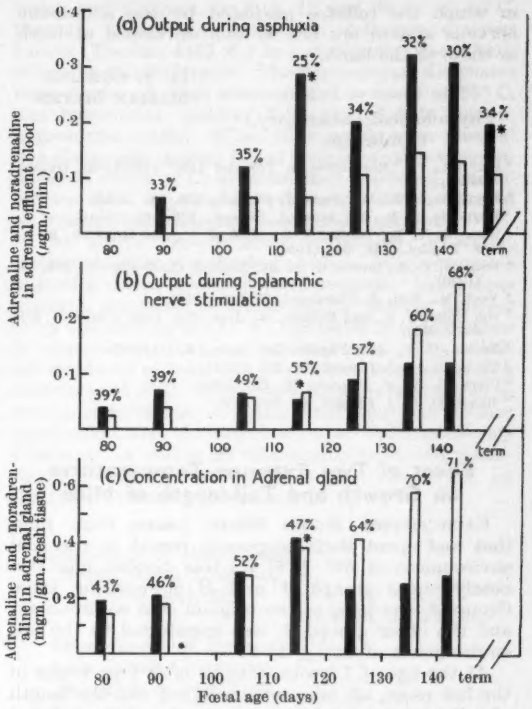

(a) Output during asphyxia
(b) Output during Splanchnic nerve stimulation
(c) Concentration in Adrenal gland
Adrenaline and noradrenaline in adrenal effluent blood (μgm./min.)
Adrenaline and noradrenaline in adrenal gland (mgm./gm. fresh tissue)
Fœtal age (days)
term

Fig. 1. Adrenaline and noradrenaline output and content of fœtal adrenal glands in the sheep. The percentage of adrenaline is given above each histogram. Black, noradrenaline; white, adrenaline. * One experiment: other figures are means of two or more experiments

in the fœtal adrenal effluent blood. Both adrenaline and noradrenaline were released, but noradrenaline was invariably predominant, irrespective of the age of the fœtus (Fig. 1, *a*). This discharge from the fœtal adrenal medulla continued until the fœtal heart stopped which, in the majority of experiments, was about 10–15 min. after ligation of the umbilical cord.

The characteristically high concentration of noradrenaline in the fœtal adrenal blood during asphyxia is in marked contrast to the response obtained during stimulation of the left splanchnic nerve. With this stimulus the ratio of adrenaline to noradrenaline in the adrenal effluent blood corresponded to that in the gland. Thus when, in the last part of pregnancy, adrenaline became the major sympathomimetic amine in the adrenal gland of the fœtal lamb, it also became the dominant amine in the adrenal effluent blood during stimulation of the splanchnic nerve (Fig. 1, *b*, *c*).

The left splanchnic nerves were cut in the present experiments, and thus the response of the fœtal adrenal to asphyxia cannot be attributed to a reflex discharge similar to that reported in the adult[7,8]. Only a trace of sympathomimetic amines was detected in arterial blood samples taken after umbilical cord occlusion. It is therefore improbable that the right adrenal of the fœtus, which retained its splanchnic innervation, contributed to the high concentration of these amines in the effluent blood from the left gland. This sensitivity of the fœtal adrenal medulla to asphyxia may be particularly important in those species, such as the cat and dog[9,10] and the rabbit[11],

in which the reflexes mediated by the autonomic nervous system are not as well developed at birth as those of the lamb.

R. S. Comline
Marian Silver

Physiological Laboratory,
Cambridge.

[1] Barcroft, J., "Researches in Prenatal Life" (Blackwell, Oxford, 1946).
[2] Reynolds, S. R. M., *Amer. J. Physiol.*, **176**, 162 (1954).
[3] Reynolds, S. R. M., *Amer. J. Physiol.*, **176**, 169 (1954).
[4] Born, G. V. R., Dawes, G. S., Mott, J. C., and Renwick, B. R., *J. Physiol.*, **132**, 304 (1956).
[5] Born, G. V. R., Dawes, G. S., and Mott, J. C., *J. Physiol.*, **134**, 149 (1956).
[6] Vogt, M., *Brit. J. Pharmacol.*, **7**, 325 (1952).
[7] von Euler, U. S., and Folkow, B., *Arch. Exp. Path. Pharmak.*, **219**, 242 (1953).
[8] Rapela, C. F., *Acta Physiol. Lat. Amer.*, **6**, 1 (1956).
[9] Clark, G. A., *J. Physiol.*, **74**, 391 (1932).
[10] Clark, G. A., *J. Physiol.*, **83**, 229 (1935).
[11] Bauer, D. J., *J. Physiol.*, **93**, 90 (1938).

Effect of Two Extreme Temperatures on Growth and Tail-length of Mice

Each of six mouse litters (taken from mice that had spent their pregnancy period in the cold environment of 50° ± 5° F.) was divided into two nearly equal groups *A* and *B* at weaning time. Group *A* was kept in the original cold environment and the other group, *B*, was transferred to the hot environment of 92° ± 1° F.

At the age of 7 weeks, that is, after four weeks in the hot room, all mice were weighed and the length of tail was measured between the tip of the tail and the point where the hair on the body ends, that is, the naked more or less hairless tail which was completely exposed to the environmental conditions.

Table 1 shows the mean body-weights of mice at weaning time and at 49 days old.

Table 1

Mice	Sex	Mean weight (gm.)	Max.	Min.
At 21 days	Males (19)	7·33 ± 1·60		
	Females (21)	7·47 ± 1·40		
Group *A**	Males (8)	20·33	24	13
	Females (11)	20·54	24	14
Group *B*†	Males (10)	15·10	20	11
	Females (8)	14·40	19	10

* After seven weeks in the cold environment.
† Three weeks in the cold environment, followed by four weeks in the hot environment.

At 49 days, the mean lengths of tail were: Group *A* (20 mice), 6·75 ± 0·65 cm.; Group *B* (18 mice), 7·92 ± 0·55 cm.

The results obtained showed that mice when raised in a cold environment grew normally, but when the environment was changed from the cold to a hot one, this growth was retarded. With regard to the effect on length of tail, it is obvious that the high temperature caused an increase in the length of tail as compared with the cold environment although both groups of mice were brothers and sisters from the same litters. This appears to be a reaction for increasing or decreasing the body surface area to facilitate either heat loss or heat conservation respectively.

M. A. El-R. Ashoub

Veterinary Hygiene Department,
Faculty of Veterinary Medicine,
University of Cairo,
Giza. Oct. 29.

An Acid-fast Bacillus isolated from the Lungs of the Cape Hyrax, *Procavia capensis* (Pallas)

In August 1954 a large epitheloid granuloma containing acid-fast bacilli was observed in a lung from Cape hyrax (locally known as 'dassie'). This particular animal had been received from New Bethesda, a small village in the north-eastern region of the Cape Province.

A further 86 'dassies' from the New Bethesda district were examined, and granulomatous lesions were found in the lungs of four animals. The infected animals all came from the same farm. Material from these lungs was injected intraperitoneally and subcutaneously into further 'dassies'. One of these animals developed a caseous inguinal lymph gland containing numerous acid-fast bacilli. This caseous material was planted on various culture media, and after four months one of us (G. B.) succeeded in isolating an acid-fast organism in pure culture on Dorset's egg medium.

Morphology and cultural characteristics. The isolated organism is a Gram-positive, acid-fast, non-motile, slender, straight or slightly curved rod, measuring approximately 1·5–2μ × 0·3–0·5μ. Much's granules are often observed. It is usually possible to differentiate microscopically the 'dassie bacillus' from the vole bacillus, particularly in artificial culture.

The organism grows slowly on ordinary mycobacterium media, although Dorset's egg medium is superior. The addition of 5 per cent glycerin does not benefit the organism. It grows better at 37° C. than at 26° C. Visible growth in primary culture usually occurs after 3–4 weeks incubation, whereas in subculture it may develop in approximately a week.

The colonies are of two types; one is small, round, raised, matt, regular, almost white; the other type is considerably larger, flat, irregular and with a raised centre. The differentiation is particularly marked after 2–3 months of incubation.

It survives without subculturing for at least a year. It is sensitive to streptomycin, rimifon and *p*-amino-salicylic acid.

Biological features. Saline suspensions of the culture were inoculated into 'dassies', rabbits, guinea pigs, white rats, white mice, and several varieties of veldt rodents. The lesions produced in these animals were very similar to those produced by the vole bacillus[1] (*Mycobacterium muris*, Wells, 1937). In view of this[2], series of *Mystromys albicandatus*, guinea pigs and white mice were inoculated with comparable doses of vole bacillus and 'dassie bacillus' respectively. The lesions, after intramuscular inoculation into *Mystromys* and guinea pigs, were of two types: local abscess formation and generalization. The lesions produced by the vole bacillus were far more severe than those produced by the 'dassie bacillus', but the animals invariably recovered. In white mice, however, the vole bacillus on intravenous inoculation produced a progressive fatal pneumonia. In contrast, those inoculated with 'dassie bacillus' developed an early generalized miliary infection which regressed. The animals still appeared healthy five months later.

In conclusion, the 'dassie' bacillus shares morphological, cultural and biological characteristics with the vole bacillus. Further studies are being undertaken to determine whether the 'dassie' bacillus should be classified as an almost non-virulent variant

of the vole bacillus, or if it should be considered a new species.

J. C. Wagner
G. Buchanan
V. Bokkenheuser
Shirley Leviseur

South African Institute for Medical Research,
Hospital Street, Johannesburg.
Oct. 16.

[1] Wells, A. Q., Med. Res. Council Spec. Rep. Ser. No. 259 (1946).
[2] Grasset, E., Murray, J. F., and Davis, D. H. S., *Amer. Rev. Tuberc.*, **58**, 427 (1947).

Microbial Growth Stimulants in Spleen

Previous studies have revealed that rabbit spleen contains unidentified, water-soluble substances that are growth stimulatory for *Escherichia coli*[1], and also that beef spleen yields heat-resistant, water-soluble extractives stimulatory for species of pathogenic fungi[2]. A blood agar medium incorporating aqueous spleen infusion was formulated for the isolation of pathogenic fungi at 37° C.[2]. The stimulatory effects of spleen extracts on the growth of micro-organisms in chemically defined culture media has been investigated by us.

We have found that bovine spleen contains an active growth-factor which is highly stimulatory for the growth of a variety of micro-organisms when tested in several chemically defined culture media. Some of the species include the yeasts, *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*; the protozoa, *Ochromonas malhamensis* and *Tetrahymena pyriformis*; and the bacterium, *Lactobacillus casei*. A preliminary purification procedure of the spleen factor was accomplished by following photometrically the turbidimetric growth response of a vitamin-depleted strain of *C. albicans* in a chemically defined liquid culture medium.

One kgm. of fresh, de-encapsulated and de-fatted beef spleen was thoroughly ground and mixed with 2 l. of distilled water. The resulting mixture was infused at 50° C. for 1 hr., occasionally agitated, and then rapidly heated to 80° C. for 5 min. in order to coagulate heat-precipitable proteins. The hot mixture was filtered through a layer each of towelling, gauze and cotton. The filtrate was cooled to 10° C. to congeal any remaining fat, which was removed by filtration through paper. The latter filtrate was autoclaved at 121° C. (15 lb.) for 15 min. in order to produce additional protein coagulation. Autoclaving did not reduce the growth-stimulatory effect of the spleen infusion for *C. albicans*. One litre of aqueous crude spleen extract, which had a *p*H of 6·8, was lyophilized and 23·5 gm. of a tan amorphous powder was obtained. This powder was extracted with one litre of anhydrous methanol at 20° C. for 15 min. The methanol-insoluble residue was microbiologically inactive for *C. albicans* and therefore was discarded. The amber-coloured, methanol filtrate which contained the active principle was mixed in a separatory funnel with an equal volume of pentane (Eastman technical) at 20° C. and shaken gently and intermittently for 10 min. An inactive, white flocculent precipitate which formed in the lower methanol layer was discarded together with the inactive pentane layer. The methanol fraction was reduced in volume in an all-glass distillation apparatus *in vacuo* at 60° C. and then dried over a steam bath until dark brown solids weighing 9·36 gm. were obtained. These were dissolved in double-distilled water and dialysed for 2, 8, 12 and 36 hr. at 5° C. through transparent, seamless, cellulose dialyser tubing (Thomas 4465 A_2) in a stationary water bath of double-distilled water. The four separate dialysates were combined, then concentrated *in vacuo* at 60° C. and lyophilized, yielding 6·66 gm. of light brown, hygroscopic solids. When these solids were assayed in a chemically defined liquid medium with a vitamin-depleted strain of *C. albicans*, they produced characteristic dose response growth-curves which showed an average half-maximal value of 35 μgm./ml.

The active growth-factor is soluble in water, methanol, ethanol and other alcohols, but is insoluble in diethyl ether, chloroform and pentane. In aqueous solution, the factor is unaffected by boiling, freezing or by autoclaving at 121° C., the latter in the presence or absence of dilute hydrochloric acid. Dry solids are stable for at least one year when maintained in a desiccator at 5° C. The spleen factor is also stable in methanol solutions. Studies embracing the further purification and characterization of the stimulatory substance, as well as its relationship to known water-soluble vitamins, are in progress. These will be described in later publications.

M. L. Littman

Department of Microbiology,
Mount Sinai Hospital,
New York 29.

Michael A. Pisano

Department of Biology,
St. John's University,
Brooklyn 6, New York.

[1] Stapleton, G. E., Sbarra, A. J., and Hollaender, A., *J. Bact.*, **70**, 7 (1955).
[2] Littman, M. L., *Amer. J. Clin. Path.*, **25**, 1148 (1955).

Amino-acid Composition of Sclero-protein of the Sponge *Hippospongia equina*

We have applied the method of paper partition chromatography to ascertain qualitatively the detailed amino-acid constitution of spongin, using the commercial unbleached honeycomb sponge, gathered in the eastern Mediterranean Sea. Our results differ in important respects from those of Low[1], who used this method to investigate the amino-acid constitution of the barium hydroxide hydrolysate of the sponge, *Spongia officinalis obliqua*, collected in the Gulf of Mexico.

We have examined both acid and alkaline hydrolysates of the sponge, previously washed with water and air dried. The acid hydrolysate was prepared by heating the sponge under reflux for 8 hr. with equal parts hydrochloric acid (6 *N*) and formic acid (85 per cent), while the alkaline hydrolysate was obtained by heating under reflux for 8 hr. with saturated barium hydroxide solution. The solvents and various location agents were all prepared according to Smith[2], while this author's dipping technique was also used. For final confirmation all the spots were extracted and run as one-way chromatograms against known standards.

The amino-acids identified in the acid and alkaline hydrolysates of spongin are given in Table 1. The relative intensity of the amino-acid spots is indicated by representing strong as + + + + and weak as +.

Tryptophan was confirmed with the Ehrlich reagent; tyrosine and histidine with the diazo reagent; iodinated tyrosines with ceric sulphate–

Table 1

	Alkaline hydrolysate	Acid hydrolysate	Relative intensity of amino-acid spot
Alanine	×	×	+++
γ-Aminobutyric acid	×		++
Arginine	×	×	+++
Aspartic acid	×	×	+++
Cystine	×		+
Glutamic acid	×	×	+++
Glycine	×	×	++
Histidine	×	×	++
Hydroxyproline	×	×	++++
Leucine–isoleucine	×	×	++++
Lysine	×		++
Ornithine	×		++
Phenylalanine	×	×	++++
Proline	×	×	++++
Serine	×		++
Threonine	×	×	++
Tryptophan	×		+
Tyrosine	×	×	++
3-Iodotyrosine		×	+
3 : 5-Diiodotyrosine	×		++
Valine	×	×	++

arsenious acid[3]. Two very faint spots which could not be identified appeared in the chromatograms of the acid hydrolysate after dipping in ninhydrin. Spot *A* was characterized by an R_F of 0·23 in butanol/acetic acid and 0·63 in phenol/ammonia: spot *B* gave values of 0·25 and 0·82 in the corresponding solvents. Spot *B* (see above) together with two other very faint spots *C* and *D* were also observed in chromatograms of the alkaline hydrolysate. Spot *C* had R_F values of 0·16 and 0·62 and spot *D* 0·35 and 0·73 respectively in butanol/acetic acid and phenol/ammonia.

All the amino-acids identified by earlier workers were confirmed except α-aminobutyric acid, dibromotyrosine and methionine found by Low[1]. No signs of these compounds could be detected, while oxidation of the valine spot with hydrogen peroxide failed to reveal any methionine. Contrary to Low's findings 3-iodotyrosine could not be detected in the alkaline hydrolysate, although it was identified in the acid hydrolysate. Considered together with the absence of 3 : 5-diiodotyrosine in the acid hydrolysate it is probable that the monoiodo-compound was present as an artefact.

We thank Mr. L. Nidditch, chairman, for laboratory facilities and the Brewing Industry Research Foundation for supplying quantities of ornithine and γ-aminobutyric acid.

J. Saper
W. E. White

The Laboratory,
The Ely Brewery Co. Ltd.,
Cardiff.
Oct. 29.

[1] Low, E. M., *J. Mar. Res.*, **10**, 239 (1951).
[2] Smith, I., Ph.D. thesis, University of London (1955).
[3] Bowden, C. H., Maclagan, N. F., and Wilkinson, J. H., *Biochem. J.*, **59**, 93 (1955).
[4] Dent, C. E., *Biochem. J.*, **47**, 240 (1947).

Fluorescence of the Crystalline Lens and Electroretinographic Sensitivity Determinations

It is generally assumed that the human lens acts like a colour filter in front of the retina, reducing the sensitivity in the short wave-length region of the spectrum. In isolated lenses, absorption depends both on wave-length and on the age of the subject. Comparison of sensory spectral sensitivities of normal and aphakic (lensless) eyes has yielded information on the absorption of the lens *in vivo*[1]. We have obtained comparable information, using the electroretinogram as an index when untrained subjects are tested.

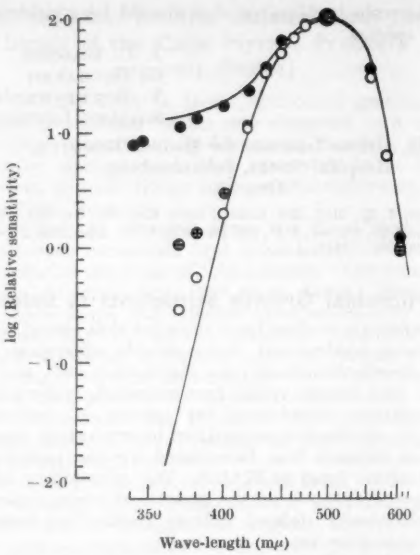


Fig. 1. Average spectral sensitivity of two aphakic eyes (filled circles), one normal eye, age 26 (open circles), and one normal eye, age 61 (crossed circles) as determined by measurement of energies producing scotopic *b*-waves of constant size (200 μV.). Spectrum of equal quantum intensity; values in terms of 497 mμ at 100 per cent. The curves represent spectral sensitivity of rod vision in an area 8° above the fovea in the normal (lower part) and aphakic (upper part) eye as determined psychophysically by Wald (ref. 1)

In order to lower the spectral limit from 400 to 341 mμ, a xenon high-pressure lamp and double-interference filters were used for stimulation. The filters were carefully selected to ensure both sufficient intensity and monochromasy (for details see ref. 2). A series of experiments was carried out on aphakic eyes of two subjects, 26 and 37 years old respectively, and on the normal eyes of two other subjects, 26 and 31 years old respectively. The average relative spectral sensitivities were calculated from the relative number of quanta eliciting *b*-waves of equal size, and their logarithms were plotted against wave-length; 497 mμ was chosen as reference standard. The results were compared with the curves for rod vision given by Wald[1] (Fig. 1).

As regards the visible part of the spectrum, there has been some discussion about the reason why sensitivity curves derived from measurements of scotopic *b*-waves are shifted towards the blue part of the spectrum[3]. Altogether, our electroretinographic determinations in the visible part of the spectrum from both normal and aphakic eyes, show rather close correspondence to Wald's curve. Peak spectral sensitivities were achieved at filter 497 mμ.

For the ultra-violet wave-length region, the values obtained from aphakic eyes fit the corresponding psychophysical curve fairly well. No differences were seen between the 26- and the 37-years-old subject respectively. However, for the young normal subject the ultra-violet sensitivity lies above Wald's curve. The corresponding values for the old normal eye

are even higher. Since the absorption of the lens for short wave-lengths is known to increase with age[1,4], lower sensitivities would be expected in the older eye. When one observes a normal eye illuminated with ultra-violet light strong enough to cause a medium electroretinogram response, the lens appears as a whitish patch in a dark surround, that is, it fluoresces. The upper limit of the spectral region where fluorescence occurs lies at short wave-lengths in children and moves to longer wave-lengths with age[5]. It is tempting to relate these facts to the observed increase of spectral sensitivities, which also depends on age. Thus, fluorescence might account for the discrepancy between electroretinogram and sensory spectral sensitivity curves respectively.

This view is supported by further measurements relating response amplitude to stimulus intensity. The slopes of the curves for normal eyes are steeper in the ultra-violet than in the visible spectrum, thus showing why Wald's relative ultra-violet sensitivities for intact eyes are lower than those reported here. His values were obtained by absolute threshold determinations, whereas electroretinogram measurements require intensities much above the absolute threshold.

This work was supported by grants from the Deutsche Forschungsgemeinschaft towards the cost of apparatus. The support of this investigation by the Department of Ophthalmology, University of Heidelberg, which referred patients with lensless eyes to us, is gratefully acknowledged.

E. Dodt
J. B. Walther

William G. Kerckhoff–
Herzforschungsinstitut
der Max Planck–Gesellschaft,
Bad Nauheim.

[1] Wald, G., *Docum. Ophthal.*, **3**, 94 (1949); *Amer. J. Ophthal.*, **40**, 18 (1955).
[2] Dodt, E., Elektroretinographie, Hamburger Symposium 1956, *Bibl. Ophthal.*, **48**, 32 (1956).
[3] Weale, R. A., *Brit. J. Ophthal.*, **40**, 392 (1956).
[4] Schanz, F., *Pflügers Arch.*, **161**, 384 (1915).
[5] Trendelenburg, W., "Der Gesichtssinn" (Berlin, Springer Verlag, 1943).

Effect of a Raised Peripheral Temperature upon the Rate of Regeneration in a Mammalian Peripheral Nerve

It has recently been reported[1] that an environmental temperature close to freezing point inhibited nerve regeneration in the rat's caudal nerve after crush injury. In these experiments only the peripheral part of the nerve was cooled, the temperature of the cell body being unaltered (for details, see Gamble and Jha[2]).

Similar experiments have now been made upon ten rats kept at room temperature (21–22° C.) and upon six kept in heated cages (35° C.). In these conditions the body temperatures are the same, but the subcutaneous temperatures in the tail differ. In the animals kept at 21–22° C. the subcutaneous temperature in the tail at a level close to the nerve injury was found to lie between 27° C. and 30° C. In the animals kept at 35° C., subcutaneous temperatures in the tail ranged between 32° C. and 36° C. It is likely, however, that during much of their sojourn in the heated cages subcutaneous temperatures of these animals lay between 35° C. and body temperature (36–39° C.).

Where the caudal temperature lay between 27° and 30° C. only equivocal evidence of remyelination was found when survival lasted 15–19 days after nerve crush (five animals). Many fibres showed double, wavy osmophilic lines, suggestive of cylinders of rather variable calibre, separated longitudinally by gaps of variable extent. It seemed reasonable to suppose that these cylinders represented very early stages in the process of remyelination. It was not until after 21 days survival, however, that unequivocal evidence of remyelination was obtained (three specimens). Nodes of Ranvier were distributed evenly along the majority of the fibres, the internodal lengths being short and uniform in all fibres. This condition was found after only 12 days (one specimen) and 14 days (three specimens) where the subcutaneous temperature lay close to 35° C.; evenly spaced nodes of Ranvier at short intervals again provided unequivocal evidence of remyelination in the majority of the fibres. After less than 12 days in the heated cages, only equivocal evidence of remyelination was found.

It is concluded that raising the temperature of the tissues through which regenerating axons pass by some 5–10° C. (from 27–30° C. to 35–37° C.) considerably speeds the earlier stages in the remyelination of those fibres. Other work in progress in this Department suggests that this effect is continued into later maturation stages.

The lower temperatures involved in these experiments lie well within the range found both subcutaneously and more deeply in the distal parts of the limbs of man. A proximodistal temperature gradient in the limbs might be related to Sunderland's[3] findings that, in the longer human nerves, regeneration after injury is more rapid in the proximal than in the distal parts. It seems possible, at least, that human nerve injuries might respond to heat treatment of a very simple kind by acceleration of the growth and maturation of regenerating fibres.

H. J. Gamble

Dept. of Anatomy,
St. Mary's Hospital Medical School,
(University of London),
Paddington, London, W.2.
Nov. 1.

[1] Gamble, H. J., *Nature*, **180**, 146 (1957).
[2] Gamble, H. J., and Jha, B. D., *J. Anat.*, (in the press).
[3] Sunderland, S., *J. Neurol. Psychiat.*, **56**, 133 (1946).

A Method for studying separately the Properties of the Innervated and Non-innervated Membrane of an isolated Single Electroplax of the Skate

The cellular unit of the electric organ of the skate (*Raja eglanteria*) is morphologically similar to that of *Electrophorus electricus*: both have rather large dimensions, although the electroplax of the skate is smaller and more irregular in shape; both have an innervated and a non-innervated membrane, the latter characterized by prominent digitations. There are, however, some differences of functional properties. The discharge of the organ in the intact animal has been studied by Albe-Fessard[1] and that of the individual electroplax by Brock *et al.*[2].

In view of the morphological similarity of the electroplax of the two species, it seemed worth while to try to adapt a new method recently developed for the single isolated electroplax of *Electrophorus* to the

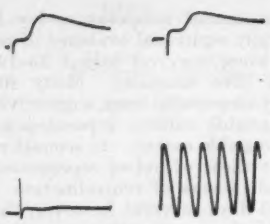


Fig. 1. Discharge of a single isolated electroplax from the skate (*Raja eglanteria*) before and after application of 0·1 μmole/ml. of *d*-tubocurarine chloride to the solution bathing the innervated membrane. From upper left to lower right: control; 1 and 4 min. after application of curare. Calibration: 5 msec., 100 mV.

corresponding unit of the skate[2–5]. This method consists in keeping a single isolated electroplax between a nylon sheet containing a window adaptable to the size and shape of the cell and a grid formed by nylon threads. The electroplax separates two pools of fluid, one of them bathing the innervated, the other the non-innervated face. Ion movements across the two types of membranes may be studied separately, and the electrical activity may be recorded with either extra- or intra-cellular electrodes. By-passing of ions has been excluded. Electrical activity remains unchanged for 6–8 hr. This method has now been adapted with minor modifications to the isolated single electroplax of the skate.

Fig. 1 shows the electrical activity recorded with extracellular electrodes and the effect of *d*-tubocurarine chloride (0·1 μmole/ml.) in the solution bathing the innervated site. The cell discharges in response to an electric stimulus with a latency period of about 3 msec. The amplitude of the response is about 30 mV. with a duration of 15–20 msec. Curare completely abolishes the response within 4 min.

The characteristics of the response are virtually the same as described by Brock *et al.*, thus confirming their finding. The advantage of the new preparation is the possibility to test the properties of the innervated membrane independently from those of the non-innervated membrane. Since it has been shown[5,6] that the two types of membranes of the electroplax of *Electrophorus* have different properties as to electric responsiveness, resting potential, ion movements and passive permeability, a study of the functionally different innervated membrane of the skate, now shown to be possible with the new method, is of obvious interest.

This work was supported in part by the Division of Research Grants and Fellowships of the National Institutes of Health, Grant No. *B*–400, United States Public Health Service, and by the Atomic Energy Commission, Contract No. *AT*(30–1)–1503.

Ernest Schoffeniels*

Department of Neurology,
College of Physicians and Surgeons,
Columbia University,
New York.
Oct. 30.

* Fulbright Fellow from the U.S. Educational Foundation in Belgium.

[1] Albe-Fessard, D., *Arch. Sci. Physiol.*, **4**, 299 (1950).
[2] Brock, L. G., Eccles, R. M., and Keynes, R. D., *J. Physiol.*, **122**, 4P (1953).
[3] Schoffeniels, E., *Fed. Proc.*, **16**, 497 (1957).
[4] Schoffeniels, E., and Nachmansohn, D., *Biochim. Biophys. Acta*, **26**, 1 (1957).
[5] Schoffeniels, E., *Biochim. Biophys. Acta* (In the press).
[6] Schoffeniels, E., *Biochim. Biophys. Acta* (in the press).

Effect of Periodic Acid on Histochemical Aldehyde Blockades

Some histochemical investigations still in progress required the use of an aldehyde blockade that would resist treatment with periodic acid and Schiff's reagent. Aldehyde groups were developed in sections of mouse tissues by oxidation with periodic acid reagent[1], by the Feulgen hydrolysis, or by carrying out these reactions successively on the same sections. Then, after blockading with hydroxylamine reagent[2], aniline hydrochloride[3], phenylhydrazine reagent[4], or 0·4 per cent w/v 1-dimethylcyclohexane-3 : 5 dione, the preparations were treated for 5 min. with the periodic acid reagent, which was followed by Schiff's reagent (E. Gurr, Ltd.) for 15 min.

Hydroxylamine blockade failed to resist treatment with periodic acid, and the sections stained with Schiff's reagent in the same manner as controls. By varying the time of initial treatment with periodic acid it was found that the staining of blockaded preparations could not be due to generation of additional aldehyde at sites where the initial oxidation might have been incomplete; hence, it seemed that periodic acid must be liberating aldehydes from aldoximes at the sites blockaded. Since there was no staining when 0·1 *N* hydrochloric acid was substituted for periodic acid after blockade, aldehyde liberation evidently was not due to acid hydrolysis alone. In some experiments with aldehydes developed by Feulgen hydrolysis, by treating sections with hydroxylamine and periodic acid alternately in series, the blockade could be broken down and reformed repeatedly.

Blockades with aniline and phenylhydrazine were resistant to periodic acid, except in the case of elastic membranes of blood vessels, which were stained strongly with Schiff's reagent. When 0·1 *N* hydrochloric acid was substituted for periodic acid after blockade, these membranes were stained just perceptibly. In all cases, this staining of elastica could be prevented by interposing a hydroxylamine blockade before Schiff's reagent.

1-Dimethylcyclohexane-3 : 5-dione did not block the reaction of aldehydes with Schiff's reagent at all sites; but where blockade was effective it was also resistant to periodic acid.

These results may be compared with those of Lillie[5], who found that 1 per cent periodic acid could liberate aldehydes at sites previously blocked with cyanide. However, his observation that blockades with aniline and phenylhydrazine were completely resistant to periodic acid apparently needs some qualification. Furthermore, it seems that when interpreting results of the periodic acid–Schiff reaction one must now envisage that some part of the staining may arise from the action of periodic acid on compounds having reactive groups analagous with the aldehyde condensation compounds that can be attacked by this reagent.

P. H. Staple*

Department of Physiology,
Medical School,
Birmingham, 15.

* Present address: Medical Research Laboratories, Charing Cross Hospital, London, W.C.2.

[1] Hotchkiss, R. D., *Arch. Biochem.*, **16**, 131 (1948).
[2] Pearse, A. G. E., "Histochemistry, Theoretical and Applied" (Churchill, London, 1953).
[3] Lillie, R. D., "Histopathologic Technic and Practical Histochemistry" (Blakiston, New York, 1954).
[4] Lessler, M. A., *J. Histochem. Cytochem.*, **4**, 36 (1956).
[5] Lillie, R. D., *J. Histochem. Cytochem.*, **4**, 479 (1956).

A Quantitative Study of Stasigenesis in Fissipede Carnivores

In the course of quantitative evolutionary studies on the dentition of fissipede carnivores we[1] have recently published a paper utilizing two angular measurements, α measuring the relation of protoconid height and talonid length in lower M_1 and β the relation between total length and protocone height in upper P_4. Graphic representation on Cartesian co-ordinates of the two values thus obtained for a number of species shows that the relative position of the values of α and β has an important biological significance, since it reveals the general phylogenetic tendencies of the group and its constituent sub-groups, and is in close agreement with the qualitative findings of taxonomy.

We are now continuing the work utilizing linear measurements. In the lower jaw of the Fissipedia we distinguish three regions of different functional significance. *P* includes the premolar series up to the paraconid of M_1; *C* measures the small sector from here to the protoconid of the same tooth; and *T* includes the remainder of the molar series. The different types of specialization found in fissipede carnivores are reflected in the very diverse magnitudes of these three regions, and when plotted reveal a pattern of points representing the species concerned. These are distributed around a central zone with a regularity capable of calculation. The parameters of the three regions, expressed in percentages, have been plotted with triangular co-ordinates in Fig. 1.

This graph reveals some striking similarities with that obtained from the values of α and β, and, at the same time, some notable differences. The variations in the values of *P*, *C* and *T* show that the amplitude is smaller for *P*. The radiation of fissipede carnivores from the time of its inception involved variations in the regions *C* and *T*. Only two families, the Ursidae and Hyaenidae, show specialization involving variation in the value of *P*.

Palæontological research has shown that these two families are of comparatively recent origin, and that they were not differentiated in the initial phase of carnivore evolution from a basal form such as *Cynodictis*, which is considered as an ideal ancestor of the group (synthetotype) because of its generalized condition and its central position in the graph.

Progressive differentiation in accordance with the idea of cosmolysis propounded by Blanc[2] results in the belated appearance of types capable of filling new ecological niches. ('Cosmolysis' is a term proposed by A. C. Blanc to denote the process of demixture and segregation of characters in an originally homogeneous population leading to the production of progressive heterogeneity and distinct groups, with particular reference to the origin of the different races of man ('anthropolysis'), on the analogy of magmatic differentiation in the inorganic world.) This is where variations in the value of *P* became possible. This new formation of families started in the Pliocene, the period that represented the climax of fissipede evolution, after which it started on its stable phase of 'stasigenesis'[3].

This process is revealed quantitatively for the first time in our studies of the linear values of *P*, *C* and *T*.

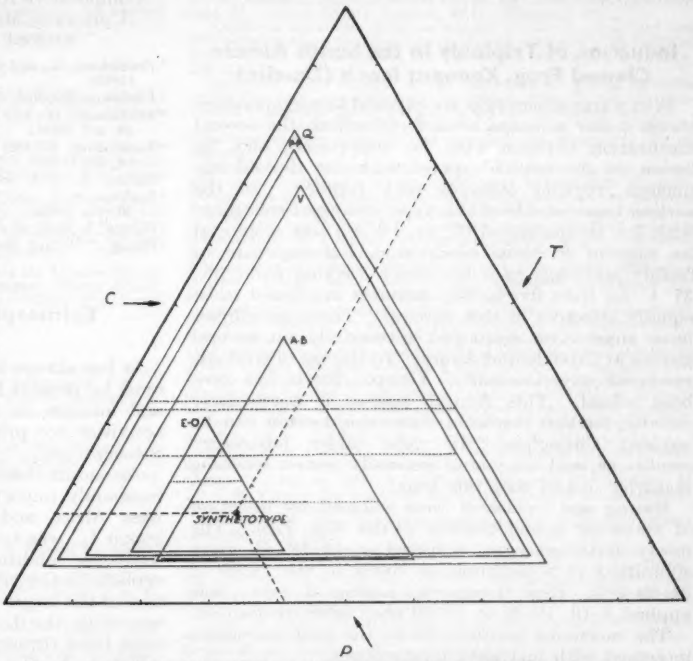


Fig. 1. Areas obtained by plotting on triangular co-ordinates of the measurements (expressed in percentages) of the lengths, *P*, premolar series to the paraconid of M_1; *C*, from the parsconid to the protoconid of M_1; and *T*, the remainder of the premolar series, in 133 species of living and fossil fissipede carnivores. The dotted lines represent the upper limits of the areas occupied by the different species. The broken lines are the co-ordinates of the synthetotype, *Cynodictis*. *E–O*, Eocene–Oligocene; *A–B*, Aquitanian–Burdigalian; *V*, Vindobonian; *P–P*, Pontian–Pliocene; *Q*, Quaternary

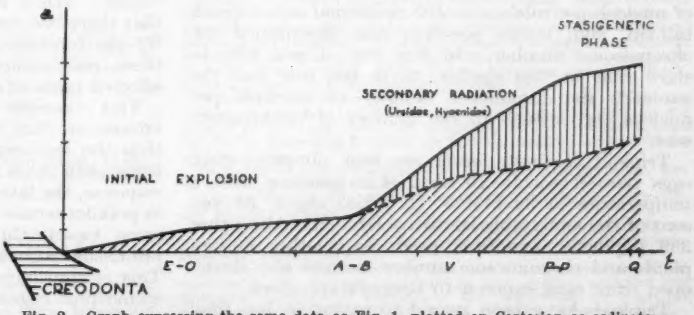


Fig. 2. Graph expressing the same data as Fig. 1, plotted on Cartesian co-ordinates. As ordinates, the amplitude of the variations expressing the increase in size of the areas of Fig. 1; as abscissæ, time expressed in absolute units. The broken line indicates the separation of the basal families from those of the Ursidae and Hyaenidae, the additive character of which is clearly shown

The rate of increase in the area shown on the graph, representing fissipede evolution, changes in the Vindobonian, and the values obtained when represented as co-ordinates plotted against time provide a graphic expression of this phenomenon (Fig. 2).

M. Crusafont-Pairó
J. Truyols-Santonja

Instituto Lucas Mallada,
Sabadell, Spain.

[1] Crusafont-Pairó, M., and Truyols-Santonja, J., *Evolution*, **10**, 314 (1956).
[2] Blanc, A. C., *Riv. Antrop.*, Rome, **34**, 179 (1943).
[3] Huxley, J. S., *Nature*, **180**, 454 (1957).

Induction of Triploidy in the South African Clawed Frog, *Xenopus laevis* (Daudin)

When amphibian eggs are exposed to a temperature shock a few minutes after fertilization, the second maturation division may be suppressed, and, by fusion of the haploid sperm with the diploid egg nucleus, triploid embryos may result[1,2]. In the earliest experiments of this type prolonged treatment with low temperatures (0° to +3° C.) was employed on eggs of *Triturus viridescens*[3], but exposure of freshly laid eggs to a hot shock varying from 35–37° C. for from five to fifty minutes was found to be equally effective in this species[4]. These techniques have since been employed successfully on several species of Urodela and Anura. To the list of triploids produced experimentally, *Xenopus laevis* has now been added. This Anuran species is particularly suitable for this research, since reproduction can be induced throughout the year under laboratory conditions, and the young are easily reared, reaching maturity in less than one year.

Mating and ovulation were induced by injection of chorionic gonadotrophin ('Ciba' *Op.* 776). The newly fertilized eggs, collected at 16–18° C., were submitted to a temperature shock in the range of 34–39·5° C. This treatment, lasting 5 min., was applied 1–10, 10–20 or 20–30 min. after oviposition. The mortality attributable to the heat treatment increased with increased temperature.

After about two days at 20–22° C. the larvæ hatched and were swimming freely 2–3 days later. On the eighth to tenth day after hatching the tail-tips were amputated and made into permanent whole preparations according to Baltzer's tail-tip technique[5].

To find the degree of ploidy, I counted the number of nucleoli per nucleus in 100 epidermal cells of each tail-tip, and, where possible, also determined the chromosome number. In this way, I was able to show that in this species, as in the frog and the axolotl[6], the maximum number of nucleoli per nucleus corresponds to the number of chromosome sets.

Triploid *Xenopus laevis* are best obtained from eggs treated within 10 min. of oviposition with a temperature of 36·1–36·5° C., when about 54 per cent of the analysable larvæ are triploid. Altogether 339 triploids have been produced. Other heteroploids and chromosome number mosaics also developed from eggs exposed to temperature shock.

Triploids have been reared to maturity, but final results concerning their growth and physiology are not as yet available. A triploid male, approximately 2½ years old when mated to a diploid female, showed greatly reduced fertility, while two other triploids of the same age, showing female characters, did not spawn after injections of large doses of hormone. Gallien[7], and Chang and Witschi[8], showed that the female is heterogametic in this species. It appears, therefore, that the triploid homogametic males can be fertile, while the heterogametic triploid females have failed so far to produce eggs.

I wish to thank Prof. R. Meier (Chemical Industry, Basle) for the chorionic gonadotrophin used in this experiment. I am very grateful to Dr. M. Fischberg for his advice and interest in this work.

Sheila Smith

Department of Zoology and
Comparative Anatomy,
University Museum,
Oxford.

[1] Fankhauser, G., and Godwin, D., *Proc. U.S. Nat. Acad. Sci.*, 34, 544 (1948).
[2] Fischberg, M., *Rev. Suisse Zool.*, **55**, 304 (1948).
[3] Fankhauser, G., and Griffiths, R. B., *Proc. U.S. Nat. Acad. Sci.*, **25**, 233 (1939).
[4] Fankhauser, G., and Watson, R. C., *Proc. U.S. Nat. Acad. Sci.*, **28**, 436 (1942).
[5] Baltzer, F., *Verh. Schweiz. Naturf. Ges.*, 248 (1922).
[6] Fankhauser, G., and Humphrey, R. R., *Proc. U.S. Nat. Acad. Sci.*, **29**, 344 (1943).
[7] Gallien, L., *Bull. Biol.*, **40**, 163 (1956).
[8] Chang, C. Y., and Witschi, E., *Proc. Soc. Exp. Biol.*, **89**, 150 (1955).

Epimorphic Regeneration during Hibernation

It has always been considered that certain factors must be present for normal epimorphic regeneration, and increase in temperature and certain metabolic activities are primary conditions which accompany regeneration.

An adult female tortoise of that species most commonly imported into Great Britain from north-west Africa and south-east Spain, *Testudo graeca graeca* L., was noted in the summer of 1954 to have a necrotic condition of the mental and first infralabial cephalic scales, this condition increasing to destruction of the larger part of the two dentary bones which constitute the distal part of the lower jaw. The tortoise lived through a spasmodic hibernation and was seriously handicapped the following summer, being prevented from normal mastication. The oral cavity was thus open to the exterior by this wound as the distal part of the lower jaw was now destroyed.

At the commencement of the second hibernation in 1956 the extent of the wound was some 4 mm. in length. While still in hibernation it was noticed that there had been closure of the ends of the wound. By the completion of hibernation two months later there was complete regeneration of the formerly affected parts of the lower jaw.

This example of epimorphic regeneration is of interest in that it occurred during hibernation and thus the temperature, oxygen and food levels were lower than those necessary for activity and voluntary response, the latter being associated with hibernation in poikilothermic animals. The total period occupied some twenty-three weeks, and of this time the pre-tissue regeneration stage took three quarters. This is approximately the time ratio for amphibian epimorphic regeneration.

Timothy J. Hunt

c/o Zoological Society of London,
Regent's Park,
London, N.W.1.

FORTHCOMING EVENTS

Monday, January 27

INSTITUTION OF ELECTRICAL ENGINEERS, RADIO AND TELECOMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Informal Evening on "An Enquiry into the Specification of Transistors". Talk by Mr. F. F. Roberts.

Tuesday, January 28

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. W. Fillingham Brown, Mr. J. C. Wylie, Mr. G. E. G. Mattingly and Mr. J. Longwell: Formal Discussion on "The Disposal of Towns' Wastes".

INSTITUTION OF ELECTRICAL ENGINEERS, RADIO AND TELECOMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Symposium on "Long-Distance Propagation above 30 Mc./s.".

WOMEN'S ENGINEERING SOCIETY, LONDON BRANCH (at the Central Electricity Authority Headquarters, Winsley Street, London, W.1), at 6.45 p.m.—Mr. C. G. Barlow: "Food Instrumentation".

Wednesday, January 29

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Prof. A. R. Ubbelohde, F.R.S.: "The Marriage of Art and Science".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. L. Douglas and Mr. A. W. Stannett: "Laboratory and Field Tests on 132 kV. Condenser Bushings".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "To What Extent Should Students be Penalized for Bad English in National Certificate Examination Papers?"

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. I. Maddock: "Ultra-High-Speed Oscillography".

Thursday, January 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. L. H. Finlayson and Prof. O. Lowenstein, F.R.S.: "The Structure and Function of Abdominal Stretch Receptors in Insects"; Mr. E. J. Denton and Miss Mary Ann Walker: "The Visual Pigment of the Conger Eel".

LINNEAN SOCIETY OF LONDON (jointly with the SYSTEMATICS ASSOCIATION, at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Meeting on "The Higher Taxonomic Group", opened by Mr. E. J. H. Corner, F.R.S., and Dr. A. J. Cain.

Friday, January 31

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Geochemical and Radiometric Methods of Surveying". Chairman: Sir William Pugh. Speakers: Mr. S. H. U. Bowie, Dr. W. Bullerwell, Dr. J. E. T. Horne and Mr. J. S. Webb.

INSTITUTION OF MECHANICAL ENGINEERS (General Meeting in conjunction with the INTERNAL COMBUSTION ENGINE GROUP, at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. W. Rizk: "Some Experimental Studies of the Mixing Processes and Flow Configuration in 2-Cycle Engine Scavenging".

INSTITUTION OF CHEMICAL ENGINEERS, GRADUATES AND STUDENTS SECTION (at Caxton Hall, Westminster, London, S.W.1), at 6.30 p.m.—Mr. P. W. Mason: "Instrumentation in the Chemical Industry".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with Brighton Technical College Chemical Society, at Brighton Technical College), at 7 p.m.—Mr. F. A. Paine: "Some Chemical Aspects of Packaging".

BRITISH GLACIOLOGICAL SOCIETY (joint meeting with the CAMBRIDGE UNIVERSITY METEOROLOGICAL SOCIETY, in the Geography Department, Downing Place, Cambridge), at 8.30 p.m.—Mr. G. Manley and Mr. C. D. Ovey: "Some Aspects of Climatic Change".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Sir Lawrence Bragg, F.R.S.: "Gemstones".

Saturday, February 1

BRITISH INTERPLANETARY SOCIETY (in the Tudor Room, Caxton Hall, Caxton Street, London, S.W.1), at 6 p.m.—Dr. N. H. Langton: "Meteoric Hazards to Space Flight".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEMONSTRATOR IN PHYSICS—The Registrar, The Manchester College of Science and Technology, Manchester 1 (January 31).

ASSISTANT PHYSICIST (with a good honours degree in physics) IN THE PHYSICS DEPARTMENT of the Sheffield National Centre for Radiotherapy—The Secretary, Sheffield Regional Hospital Board, "Broom Cross", Tree Root Walk, Sheffield 10 (February 1).

HEAD OF THE CHEMISTRY DEPARTMENT—The Registrar, College of Technology, Suffolk Street, Birmingham 1 (February 7).

MATHEMATICIAN (Assistant Experimental Officer grade), to work in the Agricultural Research Council statistical group dealing with a wide range of agricultural subjects—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (February 7).

ASSISTANT LECTURER or LECTURER IN PURE MATHEMATICS—The Registrar, The University, Nottingham (February 8).

ASSISTANT LECTURER or LECTURER IN STATISTICS—The Secretary, The University, Exeter (February 8).

DEMONSTRATOR (with a degree in agriculture) IN AGRICULTURE, for duties on the University Farms—The Registrar, The University, Leeds 2 (February 10).

ANALYST (B.Sc., B.Sc.(Agr.), or equivalent, with emphasis on chemistry) AT YANCO EXPERIMENTAL FARM, Department of Agriculture, New South Wales, for duties which include research and routine chemical work associated with rice-growing problems—The Agent-General for New South Wales, 56 Strand, London, W.C.2 (February 12).

SENIOR TECHNICIAN (A.I.M.L.T. or equivalent qualification, and preferably with experience in museum and histological techniques) IN THE DEPARTMENT OF ANATOMY, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 12).

ASSISTANT LECTURER (with an honours degree in psychology and additional training or experience in clinical psychology) IN CLINICAL PSYCHOLOGY—The Registrar, The University, Manchester 13 (February 15).

LECTURER IN PSYCHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 15).

RESEARCH ASSISTANT or JUNIOR FELLOW IN AERONAUTICAL ENGINEERING—The Registrar and Secretary, The University, Bristol (February 15).

RESEARCH BIOCHEMIST (with a university honours degree in science, with biochemistry as a major subject, plus a knowledge of microbiology and some postgraduate research experience) at the Regional Pastoral Laboratory, Division of Animal Health and Production (C.S.I.R.O.), Armidale, N.S.W., Australia, to join a team of scientists investigating the chemical, biochemical, agronomic and nutritional aspects of a general programme concerned with the utilization and management of pastures—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 208/104 (February 15).

SCIENTIFIC OFFICER (with a first- or second-class honours degree in physics, chemistry or chemical engineering) AT THE WATER POLLUTION RESEARCH LABORATORY (D.S.I.R.), Stevenage, Herts, to undertake (*a*) laboratory studies and theoretical consideration of settlement of flocculating and non-flocculating solid particles in quiescent and flowing aqueous media, and (*b*) laboratory and pilot plant studies of influence of design of sedimentation tanks on flow of liquid through such tanks and on settlement of suspended particles—The Ministry of Labour and National Service, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting F.3/8A (February 15).

U.S. RUBBER COMPANY POSTGRADUATE RESEARCH STUDENT (with a good honours degree including chemistry) to read for a higher degree, working on cationic polymerization under the direction of Dr. P. H. Plesch—The Registrar, University College of North Staffordshire, Keele, Staffs (February 22).

LECTURER IN STATISTICS—The Secretary, The University, Aberdeen (February 24).

ASSISTANT LECTURER or LECTURER (with special qualifications in mathematical statistics and some interest in industrial and engineering applications) IN THE MATHEMATICS DEPARTMENT—Head of the Mathematics Department, Imperial College of Science and Technology, London, S.W.7 (February 28).

CHAIR OF GEOGRAPHY tenable at the London School of Economics and Political Science—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 28).

DIRECTOR OF THE ZOOLOGICAL PARK, Auckland, New Zealand—New Zealand House, 415 Strand, London, W.C.2, or The Town Clerk, Town Hall, Auckland, New Zealand (February 28).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY—The Registrar, The University, Hull (February 28).

RESEARCH SPECTROSCOPISTS (2) (with a university degree of M.Sc. or Ph.D. in chemistry or physics, and preferably with some research experience in the general field of spectroscopy) IN THE DIVISION OF INDUSTRIAL CHEMISTRY (C.S.I.R.O.), Fisherman's Bend, Melbourne, Australia, to work on the following research topics: (*a*) development of the field of atomic absorption spectroscopy and its application to theoretical and applied problems, and (*b*) experimental and theoretical studies of electronic and vibrational spectra of co-ordination compounds in relation to structure—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 590/658 (February 28).

WELLCOME RESEARCH FELLOW IN ZOOLOGY at the University College of Rhodesia and Nyasaland, to collaborate in work on the physiology of tsetse flies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

LECTURER IN THE DEPARTMENT OF BOTANY—The Secretary, The University, Edinburgh (March 1).

GEORGE KELLEY READER IN METALLURGY—The Registrar, University Registry, Oxford (March 20).

ASSISTANT LECTURER (Grade "B") TO TEACH ORGANIC AND GENERAL CHEMISTRY—The Clerk to the Governors, Northern Polytechnic, Holloway, London, N.7.

LECTURER (preferably with qualifications or experience in chemical engineering and practical experience in radiochemical work) IN THE NUCLEAR TECHNOLOGY SECTION of the Department of Chemical Engineering—Prof. P. V. Danckwerts, Imperial College of Science and Technology, London, S.W.7.

METALLURGIST, CHEMIST or PHYSICIST (with an appropriate degree), for work in the Information Department dealing with every aspect of the recording and transmission of information on the extraction, manufacture and uses of non-ferrous metals and their alloys—The Secretary, British Non-Ferrous Metals Research Association, Euston Street, London, N.W.1.

RESEARCH CHEMISTS (organic chemists with physical chemistry interests, especially kinetics, or similar), for work on oxidation and ozonolytic reactions—The Director of Research, The British Rubber Producers' Research Association, 48–56 Tewin Road, Welwyn Garden City, Herts.

SENIOR LABORATORY TECHNICIAN (male, with previous experience as technician or steward in biology)—The Department of Zoology and Comparative Physiology, The University, Edgbaston, Birmingham 15.

SENIOR LECTURER IN PHYSICS; a SENIOR LECTURER IN HIGH POLYMER SCIENCE; and a LECTURER IN CHEMICAL TECHNOLOGY and/or CHEMICAL ENGINEERING—The Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in physics, and postgraduate applied research experience), to join a team of chemists and chemical engineers making fundamental and applied research studies in the inorganic chemical field—The Senior Recruitment Officer, Atomic Weapons Research Establishment, Aldermaston, Berks, quoting Ref. 1665/34.

UNIVERSITY LECTURER AND UNIVERSITY DEMONSTRATORS IN PHYSICS—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Current Medical Research. (A reprint of articles in the Report of the Medical Research Council for the year 1955–56.) Pp. iii+56. (London: H.M. Stationery Office, 1957.) 2s. 6d. net. [2510

Bishop's Stortford College Natural History Society. Annual Report. (*Coturnix* 1956–57, June 1957.) Pp. 40. (Bishop's Stortford: Bishop's Stortford College Natural History Society, 1957.) [2510

Transactions of the Royal Society of Edinburgh. Vol. 63, Part 2 (No. 15): On Protopitys (Göppert); with a Description of a Fertile Specimen *Protopitys scotica* sp. nov. from the Calciferous Sandstone Series of Dunbartonshire. By John Walton. Pp. 333–340+3 plates. 6s. Vol. 63, Part 2 (No. 16): Cytological Studies of Melaniidae (Mollusca) with Special Reference to Parthenogenesis and Polyploidy. 1: Oögenesis of the Parthenogenetic Species of *Melanoides* (Prosobranchia-Gastropoda). By Dr. Joseph Jacob. Pp. 341–352+4 plates. 6s. (Edinburgh and London: Oliver and Boyd, Ltd., 1957.) [2510

Planning, Vol. 23, No. 415 (28 October 1957): Economics of Domestic Pets. Pp. 197–212. (London: Political and Economic Planning, 1957.) 2s. 6d. [2910

Institution of Electrical Engineers. List of Members, Part 1: Members, Associate Members and Companions. Pp. 672+84. (London: Institution of Electrical Engineers, 1957.) [2910

Forestry Commission. Forest Record No. 34: Experiments on the Chemical Control of *Rhododendron ponticum*. By G. D. Holmes. Pp. 7. (London: H.M. Stationery Office, 1957.) 9d. net. [2910

The University in Africa. By Prof. H. Jac. Rousseau. (An Inaugural Lecture given in the University College of Rhodesia and Nyasaland.) Pp. 29. (London: Oxford University Press, 1957.) 3s. net. [411

International Association for the Exchange of Students for Technical Experience. Tenth Annual Report, November, 1957. Pp. 68. (London: International Association for the Exchange of Students for Technical Experience, 1957.) [411

Imperial College of Science and Technology. Vacation Training. Pp. vii+34. (London: Imperial College of Science and Technology, 1957.) [411

Annual Report of the Advisory Council on Scientific Policy, 1956–1957. Pp. 27. (Cmnd. 278.) (London: H.M. Stationery Office, 1957.) 1s. 4d. net. [411

Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1956–57. Uganda: Cotton Research Station, Namulonge; and Cotton Breeding at Serere. Pp. 51. (London: Empire Cotton Growing Corporation, 1957.) 1s. [411

Aero Research Technical Notes. Bulletin No. 178: Some Uses of 'Aerodux' 185 Resorcinol-formaldehyde Glue. Pp. 10. (Duxford, Cambridge: Aero Research, Ltd., 1957.) [411

General Register Office. Studies on Medical and Population Subjects, No. 11: Internal Migration—a Study of the Frequency of Movement of Migrants. By John A. Rowntree. Pp. ii+11. (London: H.M. Stationery Office, 1957.) 1s. 3d. net. [411

Royal Institute of Chemistry, London Section. Annual Report of the Committee, 1957. Pp. 7. (London: Royal Institute of Chemistry, 1957.) [411

Research Association of British Rubber Manufacturers. Annual Report and Accounts, 1956. Pp. 38. (Shawbury, Shrewsbury: Research Association of British Rubber Manufacturers, 1957.) [411

Iodine Information, No. 51: Iodine in the Prophylaxis of Influenza. By Dr. I. G. K. Menon and C. W. Shaw. Pp. 13. (London: Chilean Iodine Educational Bureau, 1957.) [411

British Electrical and Allied Industries Research Association. Technical Report L/T348: Debye Losses due to Electrons Trapped at Vacancies. By J. A. Sussmann. Pp. 7. 9s. Technical Report V/T 130: Electronic Voltage Stabilizers for Laboratories, Computers and Control Systems. By J. Miedzinski and S. J. Zgorski. Pp. 19+8 figures. 12s. 6d. (Leatherhead: British Electrical and Allied Industries Research Association, 1956.) [411

Atomic Energy Office. Accident at Windscale No. 1 Pile on 10th October, 1957. Pp. 26. (Cmnd. 302.) (London: H.M. Stationery Office, 1957.) 1s. 3d. net. [811

Choosing a Career. Third annual issue. Pp. 84. (London: National Union of Students, 1957.) 2s. 6d. [1211

Department of Scientific and Industrial Research. Forest Products Research. Bulletin No. 41: The Strength of Nailed Joints. By G. R. Brock. Pp. iv+32+4 plates. (London: H.M. Stationery Office, 1957.) 2s. 6d. net. [1211

The British Council. Annual Report, 1956–1957. Pp. v+96+7 plates. (London: British Council, 1957.) [1211

Other Countries

Revista Farmaceutica da Bahia, Ano 1, No. 1 (Junho, 1957). Pp. 56. (Salvador, Bahia: Universidade da Bahia, Faculdade de Farmacia, 1957.) [411

Bibliography of Scientific Publications of South and South East Asia. Vol. 1: 1955. Compiled jointly by Unesco Science Co-operation Offices for South and South East Asia. Pp. iv+188. (New Delhi: Insdoc, National Physical Laboratory, 1957.) [411

Boletin Iberoamericano de Cultura Tecnica, Vol. 1, No. 1 (Julio–Septiembre, 1957). Pp. 96. Published quarterly (not less than 350 pages per year). Annual subscription: 200 pesetas; 5 dollars. Single issues: 60 pesetas; 1.50 dollars. (Madrid: Instituto Iberoamericano de Investigaciones y Ensefianzas Técnicas, 1957.) [411

Table of Coefficients for Obtaining the Second Derivative Without Differences. By Herbert E. Salzer and Peggy T. Roverson. Pp. x+25. (San Diego, Calif: Convair-Astronautics, 1957.) [411

Regional Research Laboratory, Council of Scientific and Industrial Research, Hyderabad. Annual Report, 1956–57. Pp. xv+88. (Hyderabad: Regional Research Laboratory, 1957.) [411

Académie Royale de Belgique. Classe des Sciences. Mémoires, Tome 30, Fascicule 5: Sur le Rôle des Corrélations en Turbulence Homogène. Par Paul Janssens. Pp. 112. (Bruxelles: Académie Royale de Belgique, 1957.) 100 francs. [411

Deutsches Hydrographisches Institut, Hamburg. Jahresbericht Nr. 11 für das Jahr 1956. Pp. 80. (Hamburg: Deutsches Hydrographisches Institut, 1957.) [411

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. A.N.A.R.E. Reports. Series B, Vol. 1: Zoology. Some Jellyfish from Macquarie Island and Heard Island. By P. L. Kramp. Pp. ii+6. Interim Reports, No. 16: The Leopard Seal at Heard Island, 1951–54. By K. G. Brown. Pp. ii+34. (Melbourne: Antarctic Division, Department of External Affairs, 1957.) [411

Smithsonian Institution: Bureau of American Ethnology. Bulletin 165: Music of Acoma, Isleta, Cochiti and Zuñi Pueblos. By Frances Densmore. Pp. xii+117+6 plates. (Washington, D. C.: Government Printing Office, 1957.) 70 cents. [411

United Nations Regional Cartographic Conference for Asia and the Far East, 15–25 February 1955, Mussoorie, India. Vol. 2: Proceedings of the Conference and Technical Papers. Pp. x+133. 1.50 dollars; 11s.; 6.50 Swiss francs. International Map of the World on the Millionth Scale: Report for 1955. Prepared by the Secretariat. Pp. iii+84. 0.80 dollars; 5s. 6d.; 3.50 Swiss francs. (New York: United Nations; London: H.M. Stationery Office, 1957.) [411

Colony of Fiji. Department of Lands, Mines and Surveys: Geological Survey Section. Annual Report for the year 1956. Pp. i+9. (Suva: Government Printer, 1957.) [411

United States Department of Agriculture. Leaflet No. 417: Tobacco Cutworms—How to Control Them. By F. S. Chamberlin and Norman Allen. Pp. 8. (Washington, D.C.: Government Printing Office, 1957.) [1211

Museum of Comparative Zoology and Harvard College, Breviora. No. 80 (September 30, 1957): A Collection of Drawings of Fishes Ascribed to J. P. Kirtland (1793–1877), in the Library of Bowdoin College. By James L. Moulton. Pp. 4+3 figures. No. 81. (September 30, 1957): Contributions to a Revision of the Earth-Worm Family Lumbricidae. 1: *Allolobophora limicola*. By G. E. Gates. Pp. 14. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1957.) [1211

Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 117, No. 4: Contribution to a Revision of the Earthworm Family Ocnerodrilidae. The Genus *Nematogenia*. By G. E. Gates. Pp. i+427–445. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1957.) (1211

The Commonwealth Fund. Thirty-ninth Annual Report for the year ending June 30, 1957. Pp. xli+41. (New York: The Commonwealth Fund, 1957.) [1211

Report of the Nutrition Committee for South and East Asia, Fourth Session, Tokyo, Japan, 25 September–2 October, 1956. Pp. v+49. (F.A.O. Nutrition Meetings Report Series, No. 14.) (Rome: Food and Agricultural Organization of the United Nations; London: H.M. Stationery Office, 1957.) 2s. 6d. [1211

Uganda Protectorate. Annual Report of the Geological Survey Department for the year ended 31st December, 1956. Pp. i+40. (Entebbe: Government Printer, 1957.) Shs. 2. [1211

United States Naval Observatory. Circular No. 80: Positions, Areas, and Counts of Sunspots, February, 1957. By Winifred Sawtell Cameron. Pp. 7. (Washington, D.C.: United States Naval Observatory, 1957.) [1211

Chicago Natural History Museum. Fieldiana: Geology. Vol. 10, No. 30: A Restudy of the 1917 Eruption of Volcán Boquerón, El Salvador, Central America. By Sharat Kumar Roy. Pp. 363–382. 75 cents. Vol. 10, No. 31: The Problems of the Origin and Structure of Chondrules in Stony Meteorites. By Sharat Kumar Roy. Pp. 383–396. 50 cents. (Chicago: Chicago Natural History Museum, 1957.) [1211

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

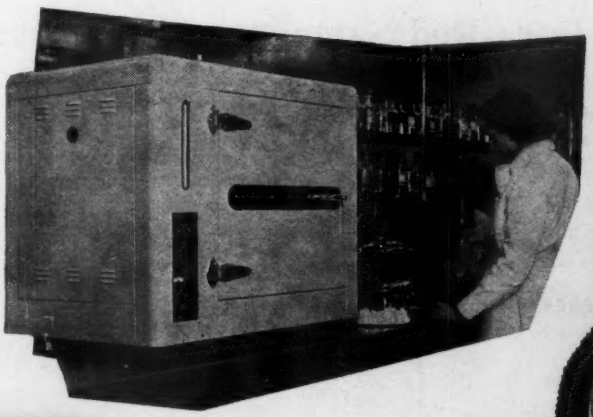
Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2
Telephone Number: Holborn 4743

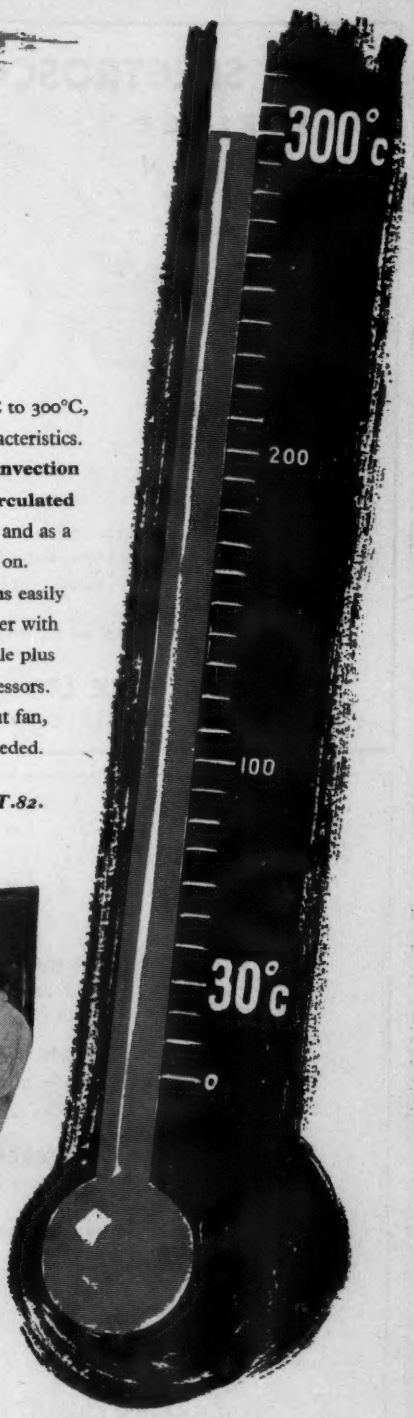
All rights reserved. Registered as a newspaper at the General Post Office

The BTL wide-range oven

This oven covers the range of 30°C to 300°C, with excellent heat-up and recovery characteristics. It can be used in three ways—as a **convection oven,** with vent open and without fan, as a **fan-circulated closed oven,** with vent shut and fan on, and as a **forced-draught oven,** with vent open and fan on. B.S. 2648 readily achieved—conditions easily reproduced for repeat tests—stainless-steel chamber with removable shelves—10″ thermo-regulator scale plus built-in thermometer—TV suppressors. The oven can be supplied with or without fan, which can easily be fitted later if needed.

Write for technical leaflet T.82.





BTL

complete laboratory service

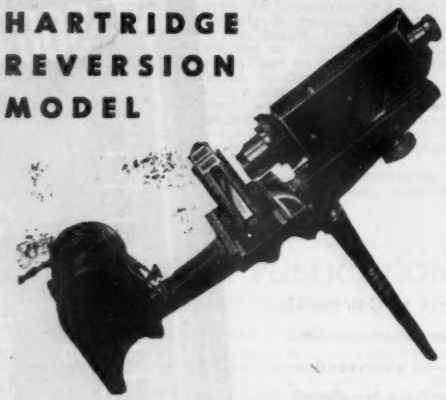
BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND.

Branches in London, Manchester and Glasgow. *Agents throughout U.K. and all over the world.*

TAS/BT.5

BECK SPECTROSCOPE

HARTRIDGE REVERSION MODEL



For all spectrometric work, absorption or emission, the reversion method gives the most accurate results. Readings can be obtained to an accuracy of 1Å.U.

Full particulars forwarded on application

R. & J. BECK LTD.
69/71 MORTIMER STREET, LONDON, W.1

FOUNDED HAWKSLEY LONDON IN 1869

THE HAWKSLEY MICRO-HAEMATOCRIT CENTRIFUGE

A new, high-speed centrifuge specially designed to give complete cell packing in five minutes using a micro quantity of capillary or venous blood.

Your usual supplier of Hawksley products will gladly give full details

HAWKSLEY & SONS LIMITED
LONDON, ENGLAND

Ultra-Violet & Infra-Red Sampling Accessories

ULTRA-VIOLET ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length cells for liquids

KBR EVACUABLE DIE
for making 13, 15 and 16 mm. Discs

INFRA-RED ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length demountable and sealed cells for liquids
MICROCELLS for liquids
GAS cells in pyrex and metal

HYDRAULIC 20-TON PRESS
especially designed for KBr Disc Technique

Rotating Electrode (Solution Excitation) Spark Unit

HIGH-SPEED VIBRATION MILL
indispensable for KBr Disc Technique
available for 230 volt 50-cycle and 110-volt 60-cycle mains

RII Sampling Accessories are marketed by the leading spectrometer manufacturers of the world. Descriptive literature on application.

RESEARCH & INDUSTRIAL INSTRUMENTS CO.
30 Langton Road, London SW9 Telephone : RELiance 6171

Printed in Great Britain by FISHER, KNIGHT & CO., LTD., The Gainsborough Press, St. Albans, and published by MACMILLAN & CO., LIMITED, at St. Martin's Street, London, W.C.2, and ST. MARTIN'S PRESS, INC., 103 Park Avenue, New York 17, N.Y., U.S.A.—Saturday, January 25, 1958